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Epidemiology of and Diagnostic Strategies for Toxoplasmosis

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INTRODUCTION

Infection with the protozoan parasite *Toxoplasma gondii* has a worldwide distribution. This obligate intracellular parasite can infect humans as well as virtually all warm-blooded animals, including mammals and birds. Since its first description in the gondi, a rodent from North Africa, by Nicolle and Manceaux in 1908 (239), the parasite was progressively recognized as the agent of a widespread zoonosis. However, its entire life cycle was definitively understood only in the late 1960s (95, 133, 176), with the discovery of the central role of the cat as a definitive host harboring the sexual parasitic cycle and spreading oocysts through feces. In the same period of time, it was classified in the coccidian subclass (133), phylum *Apicomplexa*, and the infectivity of the three parasitic stages (tachyzoite, cyst, and oocyst) was well characterized.

The true importance of toxoplasmosis in humans remained unknown until the first reports of cases of congenital toxoplasmosis (299). The history of clinical toxoplasmosis and the wide spectrum of this disease revealed over the years were reviewed by Weiss and Dubey in 2009 (336). The growing role of *Toxoplasma* infection in immunocompromised patients was acknowledged in the mid-1970s, and the concept of the reactivation of infection was thereafter extensively explored by immunologists. During the last decade, the development of new genotyping tools and the multiplication of field studies have led to breakthroughs in the comprehension of the phylogenetic evolution of *T. gondii* in the world (222), and recent advances in our knowledge of the particular virulences associated with some genotypes have been achieved (291).

In this paper, we provide an updated review of data on toxoplasmosis, with a focus on the epidemiological and diagnostic angles, putting them into perspective with current knowledge of parasite genotypes.

BIOLOGY OF THE PARASITE

Three Parasitic Stages

There are three infective stages of *T. gondii*: a rapidly dividing invasive tachyzoite, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst. These infective stages are crescent-shaped cells, approximately 5 μm long and 2 μm wide, with a pointed apical end and a rounded posterior end. They are limited by a complex membrane, named the pellicle, closely associated with a cytoskeleton involved in the structural integrity and motility of the cell. They possess a nucleus, a mitochondrion, a Golgi complex, ribosomes, an endoplasmic reticulum, and a multiple-membrane-bound plastid-like organelle called the apicoplast, the result of a possible acquisition by the parasite via a secondary endosymbiosis of a free-living red alga (285). As for other members of the phylum *Apicomplexa*, they concentrate in their apical part a specialized cytoskeletal structure (the conoid, involved in cell invasion) and numerous secretory organelles (rhoptries [ROPs], dense granules, and micronemes). More details were reported elsewhere previously (6, 101, 335).

Tachyzoites are the dissemination form (Fig. 1A). They are able to invade virtually all vertebrate cell types, where they multiply in a parasitophorous vacuole.

Bradyzoites result from the conversion of tachyzoites into a slow-dividing stage and form tissue cysts (Fig. 1B). These cysts are more or less spheroid in brain cells or elongated in muscular cells. They vary in size from 10 μm for the younger cysts, containing only two bradyzoites, to up to 100 μm for the older ones, containing hundreds or thousands of densely packed bradyzoites. The cyst wall consists of a limiting membrane presenting numerous invaginations and an underlying layer of electron-dense granular material (124). Bradyzoites have a latent metabolism, well adapted to long-term survival. Cysts remain intracellular throughout their life span. The death of the host cell may trigger the disruption of the cyst wall and the consequent liberation of bradyzoites. The resistance of bradyzoites to the acid pepsin (1- to 2-h survival into pepsin-HCl) allows their transmission through ingestion.

Sporozoites are located in mature oocysts. Oocysts are 12- to 13-μm ovoid structures that after sporulation contain two sporocysts, each containing four sporozoites (Fig. 1C and D). The oocyst wall is an extremely robust multilayer structure protecting the parasite from mechanical and chemical damages. It enables the parasite to survive for long periods, up to more than a year, in a moist environment (213).

Life Cycle of *T. gondii*

T. gondii is a tissue-cyst-forming coccidium functioning in a prey-predator system that alternates between definitive (sexual reproduction) and intermediate (asexual replication) hosts. It is unique among this group because it can be transmitted not only between intermediate and definitive hosts (sexual cycle) but also between intermediate hosts via carnivorism (asexual cycle) or even between definitive hosts. The parts of the sexual and asexual cycles and transmission dynamics in a given environment vary according to physical characteristics and according to the structures of both intermediate and definitive host populations (4).

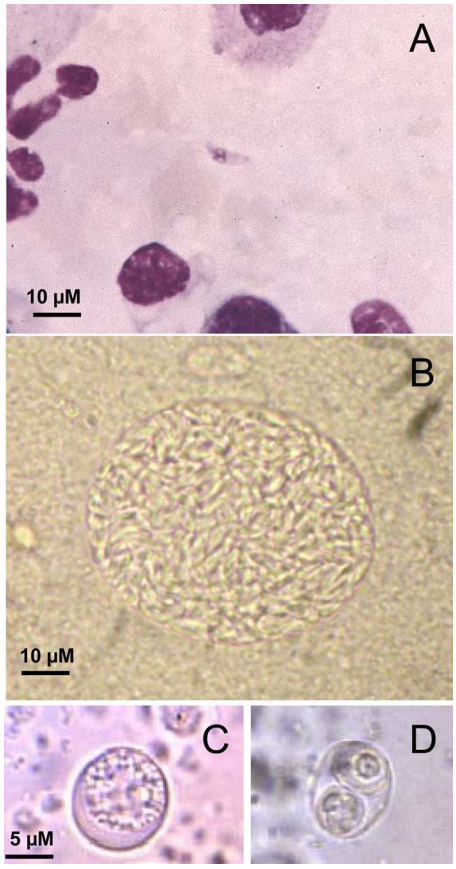

FIG 1 Biologic stages of *Toxoplasma gondii*. Shown are microscopic examinations of tachyzoites in a bronchoalveolar lavage fluid sample stained with Giemsa (A) (magnification, ×500), a cyst in the brain of an infected mouse (B) (magnification, ×500), and unsporulated (C) and sporulated (D) oocysts (magnification, ×1,000).

Sexual reproduction occurs only in felids (domestic and wild cats). After the ingestion of cysts present in tissues of an intermediate host, the cyst wall is destroyed by gastric enzymes. Bradyzoites settle within enterocytes, where they undergo a self-limiting number of asexual multiplications, characterized by the development of merozoites within schizonts (Fig. 2) (90). This first step is followed by sexual development, with the formation of male and female gametes (gametogony) (123). After fertilization, oocysts formed within enterocytes are liberated by the disruption of the cell and excreted as unsporulated forms in cat feces (Fig. 2). The process of sporogony occurs after a few days in the external environment. It implies a meiotic reduction and morphological changes leading to the formation of a sporulated oocyst with two sporocysts, each containing four haploid sporozoites. The shedding of oocysts begins 3 to 7 days after the ingestion of tissue cysts and may continue for up to 20 days. Infected cats can shed more than 100 million oocysts in their feces (95, 180). They can infect a wide range of intermediate hosts, virtually all warm-blooded animals, from mammals to birds, when ingested with food or water. Oocysts are also infective for cats although less efficiently.

Within intermediate hosts, the parasite undergoes only asexual development. After oocyst ingestion, sporozoites are liberated. They penetrate the intestinal epithelium, where they differentiate into tachyzoites. Tachyzoites rapidly replicate by endodyogeny inside any kind of cell and disseminate throughout the organism. As a result of the conversion from tachyzoite to bradyzoite, tissue cysts arise as early as 7 to 10 days postinfection and may remain throughout life in most hosts, predominantly in the brain or musculature.

Upon the ingestion of these tissue cysts by an intermediate host through raw or undercooked meat, cysts are ruptured as they pass through the digestive tract, causing the release of bradyzoites. The bradyzoites will infect the intestinal epithelium of the new host and differentiate back into the rapidly dividing tachyzoite stage for dissemination throughout the body (Fig. 2).

In addition, if the acute phase occurs during pregnancy, the parasite can cross the placenta and infect the fetus (congenital transmission). A role for this vertical transmission in maintaining high levels of infection in some species has been suggested (112).

Mechanism of Cell Invasion

T. gondii is remarkable in its ability to invade a wide variety of host cells. Invasion is an active process relying on parasite motility and the sequential secretion of proteins from secretory organelles, the micronemes, the rhoptries, and the dense granules.

Attachment to the host cell membrane is a prerequisite for invasion. It requires the calcium-dependent secretion of adhesins from micronemes, such as the microneme protein MIC2, which recognize host cell receptors and promote parasite reorientation and attachment. Cell invasion relies on a complex interaction between the host cell surface and the parasite, a process called gliding motility, an intricate linear motor system promoted by actin-myosin interactions and dynamic rearrangements of the parasite cytoskeleton (50). Entry is a rapid process (15 to 30 s) distinct from currently known host endocytic events. *Toxoplasma* forms a tight association between its apical end and the host cell membrane, called the moving junction. This moving junction moves from the apical end to the posterior end of the parasite, leading to the internalization of the parasite into a parasitophorous vacuole (PV). The establishment of this moving junction around the invading parasite requires the distribution over the entire surface of the parasite of an apical membrane antigen (AMA1), also secreted by micronemes, and the secretion of rhoptry (ROP) neck proteins (RONs) inserted into the host cell membrane (108). The formation of the nascent parasitophorous vacuole membrane (PVM) requires the secretion of proteins from the ROPs. In recent years, a major role for the ROP2 family proteins has been recognized. Of these proteins, ROP18 is associated with the cytosolic face of the PVM and exerts protein kinase activity, which has a profound effect on parasite growth and virulence (116), and ROP16 is able to manipulate host gene expression, affecting interleukin secretion (197).

Besides ROP proteins, dense granular proteins also contribute to the formation of the PVM during the first hour following invasion. Most host transmembrane proteins are stripped from the PVM during the invasion process; this process modifies biochemical characteristics of the PVM and prevents fusion with lysosomes or any cytoplasmic vesicle. Dense-granule secretions also support the development of a complex network of membrane tubules that develop from the PVM and extend into the vacuolar lumen (224). This network is supposed to have a role in developing exchanges between the parasite and the host cell, bringing in nutrients from the host cell cytosol or exporting proteins or lipids from the parasite toward the PVM or the host cell. The PVM is also closely

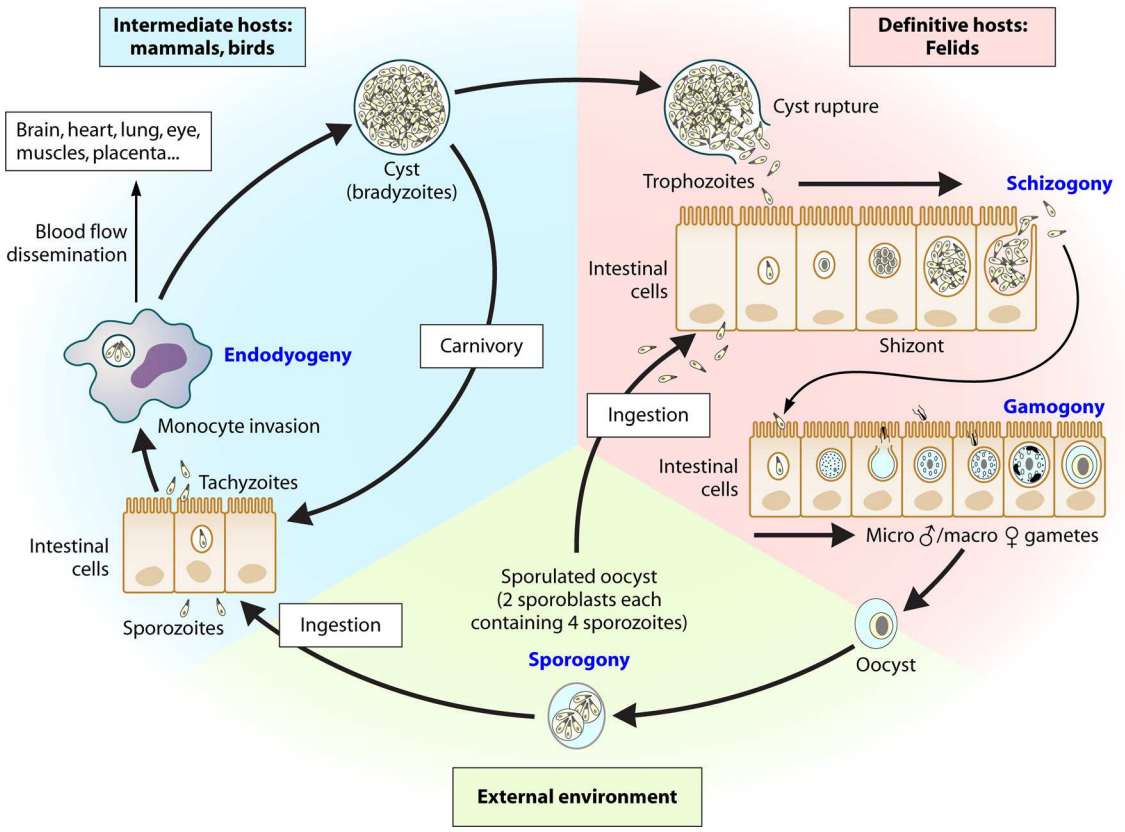

FIG 2 Life cycle of *Toxoplasma gondii*. Shown are the biology, infection, and replication of the three infective stages of the parasites in their respective hosts.

associated with host cell mitochondria, which contribute to parasite metabolism. Within the PV, tachyzoites divide during a 6- to 9-h cycle, by a process of endodyogeny, leading to the formation of two daughter cells within each mother cell. They exit the cell usually after 64 to 128 parasites have accumulated in the PV (36). Egress from the cell is an active process dependent upon a rise in the calcium concentration after the release from intracellular stores (301).

PREVALENCE AND IMPORTANCE OF TOXOPLASMOSIS IN THE WORLD

Prevalence in Animals

Methods for screening. The detection of chronic infection with *T. gondii* in animals relies primarily on serological assays. There is no gold standard test for the screening of the large diversity of *Toxoplasma* host species. The sensitivity and specificity of the techniques depend on the animal species, and cutoff values are difficult to establish because reference sera from experimentally infected animals are lacking. Even when these sera are available for one species, they may not reflect natural conditions, as experimental animals are often infected with high doses and sometimes through unnatural routes, which may induce excessively high antibody titers. Currently, the modified agglutination test (MAT) seems to be the test most adapted to a large number of species (93), but specific enzyme-linked immunosorbent assays (ELISAs) have been developed for some domestic animal species. These serological tests were first developed for serum analysis but have been adapted for the analysis of meat juice for evaluating the risk of *Toxoplasma* in meat (105, 162). The analysis of meat juice is less sensitive but is the only means to detect *Toxoplasma* antibodies when sera are not available (retail stores).

Serological surveys alone do not provide information about the prevalence of viable parasites. Indeed, *Toxoplasma* strains have been isolated from seronegative animals (96), and on the other hand, the direct detection of the parasite is frequently negative in seropositive animals (243). One of the most sensitive means of detecting cysts in animal tissues relies on bioassays. For mouse bioassays, tissues are digested *in vitro* with acid, pepsin or trypsin, prior to inoculation into mice, which are further monitored for the development of illness and seroconversion (92). Bioassays with cats are more sensitive but also more expensive than the mouse bioassay. This assay consists of feeding the cat with tissue samples and then examining the feces for the shedding of oocysts from 3 to 14 days postinoculation (93). These bioassays are laborious and time-consuming techniques that are poorly adapted to the screening of large numbers of samples. Therefore, PCR-based methods have been developed to detect parasite DNA in meat samples. However, these methods are in fact less sensitive than bioassays due to the inhomogeneous distribution of tissue cysts and due to the small size of the tissue sample used (usually 50 mg of sample for PCR assays, versus 50 to 500 g for bioassays). In an attempt to increase the sensitivity of detection by PCR, a method based on sequence-specific magnetic capture of *T. gondii* DNA followed by DNA amplification has been developed. It allowed the testing of 100-g tissue sample homogenates, with an estimated detection limit of approximately 1 cyst per 100 g (242).

Wildlife. *Toxoplasma* infection has been described for more than 350 host species, mammals and birds, with the vast majority of them living in a wild environment (114, 209, 316). The contamination of environment, and, hence, of intermediate wild hosts, is linked to the shedding of oocysts by felids, either stray or domestic cats close to farms or wild felid species. Evidence of infection (mostly by serological detection and more rarely by oocyst or tissue cyst detection) has been proven for 31 of the 39 felid species in the world (93). The seroprevalence in wild felids is usually very high and may reach up to 100%. The prevalence in the intermediate hosts depends on the presence of these felids in their environment. However, the processes promoting infection in wild-animal populations are highly complex and involve the interaction of physical, biological, and ecological characteristics, including (i) climate characteristics, where areas with dry and hot climates, unfavorable for oocyst survival, are associated with lower prevalences in wild animals, whereas the highest prevalences of infection in wildlife are found in humid tropical countries; (ii) the susceptibility of the host species to *Toxoplasma* infection (some species may be resistant or may spontaneously clear infection); (iii) the size and weight of the animal species, which are usually correlated with the duration of life, thereby influencing the chance of infection and partly explaining the low rate of infection (only 1 to 5%) of small rodents (*Mus musculus*) in most survey studies (5, 67); and (iv) the diet and feeding behavior of the host species, where the prevalence of infection is often lower in herbivores than in omnivores and carnivores due to the cumulative efficacy of the predator-prey cycle of *T. gondii* (306). Among wild mammals from the Amazonian forest (French Guiana), terrestrial mammals were significantly more exposed to *T. gondii* than arboreal mammals as a result of ground-dwelling behavior and/or carnivory (87). In the Northern Hemisphere, a high prevalence was found in carnivorous (black bears and red foxes) or in omnivorous species, such as wild boars, which are exposed to infection through the ingestion of both oocysts and tissue cysts. An ecological approach to studying the circulation of the parasite in wildlife includes studies of factors such as the migration of birds, fragmentation of the landscape (by rivers, roads, cultivated areas, and villages, etc.), dispersion of oocysts, or predation behavior of the different felid species in various environments.

Special interest has recently been focused on marine mammals. A variety of marine mammals (sea otters, dolphins, seals, and walruses) has been found to be infected, with prevalences ranging from 47 to 100%. These marine mammals serve as sentinels of environmental contamination by oocysts via freshwater runoff into the marine ecosystem (60).

Meat-producing animals: differences according to breeding. The risk of *Toxoplasma* infection in livestock has been considerably reduced by the use of intensive farm management with adequate measures of hygiene and confinement, such as keeping meat-producing animals indoors throughout their lifetimes; keeping the sheds free of rodents, birds, and cats; and feeding meat-producing animals on sterilized food (191). This has led to a marked drop in the *T. gondii* seroprevalence in pork. Seroprevalence in slaughter pigs is now <5% in most industrialized countries (99). In 2005, a nationwide survey of *T. gondii* in meat (chicken, beef, and pork) obtained from retail stores in the United States found a seroprevalence of only 0.57% in pork (97). This declining seroprevalence in pigs has been found in every industrialized country, but the recent trend of "animal-friendly" or "organic" outdoor production systems increases the exposure of pigs to a contaminated environment (119a, 316).

Prevalences in poultry also vary markedly according to production systems. *Toxoplasma* infection in industrialized poultry farms is practically absent, while the seroprevalence in free-range or backyards chickens is usually high, up to 100%. Due to their habit of feeding close to the ground, free-range chickens are indeed considered a good indicator of environmental contamination by *Toxoplasma* oocysts (93).

The prevalence in other meat-producing animals, such as sheep, goats, and horses, has not changed over time, because the source of infection of these herbivorous animals kept on pastures has remained unchanged. In farmed sheep, the seroprevalence in Europe is logically correlated with age, increasing from lambs (17 to 22%) to adult (65 to 89%) (162). Viable *T. gondii* organisms have been recovered from as many as 67% of sheep samples. Sheep, rather than pigs, are the main source of infected meat in Southern European countries. Rates of seropositivity reported for goats vary from 4 to 77% (105, 316), while they are generally lower in horses (93).

Toxoplasma infection in cattle is still a matter of debate. The reported seropositivity of cattle ranges from 2 to 92% (316). Higher infection rates are observed in calves during their first grazing season, indicating that calves become infected after exposure to *Toxoplasma* on pastures (243). Seropositivity rates decline in older animals. Despite high rates of seropositivity reported in some studies, the parasite has been detected very rarely in tissues of adult cows and in aborted fetuses (99). For example, of 2,094 samples of retail beef meat in the United States, no *Toxoplasma* infection was detected (97). This finding means that seroprevalence cannot be used as an indicator of the number of cattle carrying infectious parasites. The resistance of cattle to *Toxoplasma* infection and the ability of these animals to clear the infection have been suggested (107, 243).

Prevalence of Infection in Humans

It is generally assumed that approximately 25 to 30% of the world's human population is infected by *Toxoplasma* (230). Actually, the prevalences vary widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region (246). Low seroprevalences (10 to 30%) have been observed in North America, in South East Asia, in Northern Europe, and in Sahelian countries of Africa. Moderate prevalences (30 to 50%) have been found in countries of Central and Southern Europe, and high prevalences have been found Latin America and in tropical African countries.

As for animals, many factors can affect seroprevalence in humans. Climatic factors affecting the survival of oocysts in the environment and, hence, infection rates in meat-producing animals play a major role. Higher prevalences are classically observed for tropical countries with a humid and warm climate, and conversely, lower prevalences are found for arid countries or for colder countries, but anthropogenic factors explain a large part of the variations in human seroprevalence, including dietary habits (method of cooking meat, hand washing, kinds of meat or vegetables consumed, and vegetable cleaning, etc.); economic, social, or cultural habits; quality of water; and sanitation coverage. Seroprevalence increases with age, but the rate of acquisition of infection in relation to age varies according to the country and socioeconomic level. Near-maximal seroprevalence may be reached in

childhood in populations living under poor-hygiene conditions, probably linked to telluric or waterborne contamination by oocyst ingestion. This points toward water as an important source of human infection in areas where humans use unfiltered surface water for consumption and probably also in areas where there is contact with freshwater, for instance, for recreation (20, 117, 180). As an example, in a city located in the northern Rio de Janeiro state (Brazil), the age-adjusted seroprevalence was 84% for the group of the lower socioeconomic level, compared to seroprevalences of 62% and 23% for the groups of the middle and upper socioeconomic levels, respectively (20). Most persons (up to 84%) in the population of the lower socioeconomic level were infected by the age of 15 years, whereas infection was acquired mostly after the age of 20 years in the population of the upper socioeconomic level (from about 20% for the age group of 20 to 29 years to 70% for the age group of 40 to 49 years). In a multivariate risk factor analysis, this was attributed to differences in water supply, with the poorest populations living in areas supplied with unfiltered water. These different patterns of *Toxoplasma* acquisition according to socioeconomic levels may be more relevant in underdeveloped tropical countries, but in the United States, *Toxoplasma* infection was also considered an infection associated with poverty (174). The overall seroprevalence (U.S.- and foreign-born individuals combined) was higher among non-Hispanic black persons and Mexican Americans than among non-Hispanic white persons (181). Logically, increased socioeconomic levels, together with an improvement of hygienic conditions, changes in farming systems, the consumption of frozen meat, and the feeding of cats with sterilized food, have led to a continuous decrease of the seroprevalence in most industrialized countries over the last decades. In the United States, a national survey found a decrease in the age-adjusted *T. gondii* prevalence in U.S.-born persons aged 12 to 49 years, from 14.1% in 1988 to 1994 to 9% in 1999 to 2004 (181). In France, the seroprevalences in pregnant women were about 80% in the early 1960s, around 66% in the 1980s, 54% in 1995, and 44% in 2003, while at the same time, the average age of pregnant women increased (328). This declining seroprevalence has been observed in all areas where it was studied in Europe. For example, in The Netherlands, the seroprevalence decreased from 35.2% in 1995 to 1996 to 18.5% in 2006 to 2007 in women of reproductive age (170).

HOW DO HUMANS BECOME INFECTED?

The majority of horizontal transmissions to humans is caused either by the ingestion of tissue cysts in infected meat or by the ingestion of soil, water, or food contaminated with sporulated oocysts derived from the environment or, less frequently, directly from feline feces (Fig. 3). The relative importance of transmissions via tissue cysts versus oocysts in a given population is unknown, except in the case of outbreaks with a well-defined source of infection. Until now, only risk factor studies gave an indication of the predominant route of transmission in a given population. However, in these epidemiological studies, risk factors for infection remained unexplained in 14 to 49% of cases (61, 179). Persons may be unaware of their exposure or may have difficulty recalling specific risks that occurred. The recent discovery of a sporozoite- or oocyst-specific protein, which elicited antibody production and differentiated oocyst- versus tissue cyst-induced experimental infection in pigs and mice, may help to solve this problem (168). Serum antibodies to the sporozoite protein were detected in humans within 6 to 8 months of an initial oocyst-acquired infection. Therefore, this serological assay could be useful for detecting exposure to oocysts in the early months after *T. gondii* infection and could be useful for epidemiological studies.

Infection through Cysts

Consumption of meat. (i) Type of meat. Any meat from warm-blooded animals and birds has been traditionally considered a major source of *Toxoplasma* infection in Western countries. The risk associated with the type of meat (lamb, pork, and beef, etc.) varies among different countries according to local eating habits and according to the prevalence in meat-producing animals. In a multicenter study in Europe, meat consumption was estimated to be responsible for 30 to 63% of cases of infection, while soil contact represented 6 to 17% of cases (61). In the United States, a recent case-control study showed an elevated risk for *T. gondii* infection in persons eating raw ground beef (adjusted odds ratio [aOR], 6.67; attributable risk [AR], 7%); eating rare lamb (aOR, 8.39; AR, 20%); eating locally produced cured, dried, or smoked meat (aOR, 1.97; AR, 22%); or working with meat (aOR, 3.15; AR, 5%) (179). Outbreaks due to the consumption of undercooked meat have been described. These outbreaks generally involved only a few patients (2 to 20 persons) (reviewed in reference 5a).

A quantitative assessment of the risk of *Toxoplasma* in food for consumers is hampered by the lack of data on the number of tissue cysts resulting in infection of humans, the distribution and the number of cysts in the different muscle sites in various hosts, as well as their infectivity in commercial meat products. One recent survey of meat from commercial markets (pork, chicken, and beef) in the United States suggested a low risk, perhaps owing to meat treatment processes, which could reduce the viability of cysts (97). This may not be the case in countries where lamb and sheep are the most consumed meats (31, 162).

(ii) Cyst resistance. Tissue cysts remain infectious in refrigerated carcasses (1°C to 6°C) or minced meat for up to 3 weeks. Freezing alone is not a reliable means of rendering all tissue cysts noninfective: cysts have remained viable for >11 days at −7°C. However, the deep-freezing of meat at −12°C or lower for at least 3 days is usually efficacious to kill cysts, although it may depend on the thickness of the piece of meat (91).

Tissue cysts are usually killed immediately by heating to 67°C. The survival of tissue cysts at lower temperatures depends on the duration of cooking. Tissue cysts remain viable at 60°C for about 4 min and at 50°C for about 10 min (100). Cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *Toxoplasma* in all parts of the meat. Some tissue cysts will remain infectious after cooking in a microwave oven, possibly due to an uneven heating of the meat. However, in a U.S. case-control study (179), microwave cooking of meat was associated with a reduced risk of recent *T. gondii* infection. This was explained by the fact that microwave cooking is often associated with reheating already-cooked meat or with defrosting or cooking frozen meat.

Commercial procedures of curing with salt, sucrose, or low-temperature smoking may kill tissue cysts, but the survival time of tissue cysts varies greatly with the concentration of the salt solution and the temperature of storage. Salting does not necessarily kill tissue cysts in homemade pork sausages. Under laboratory conditions, solutions containing 2% sodium chlo-

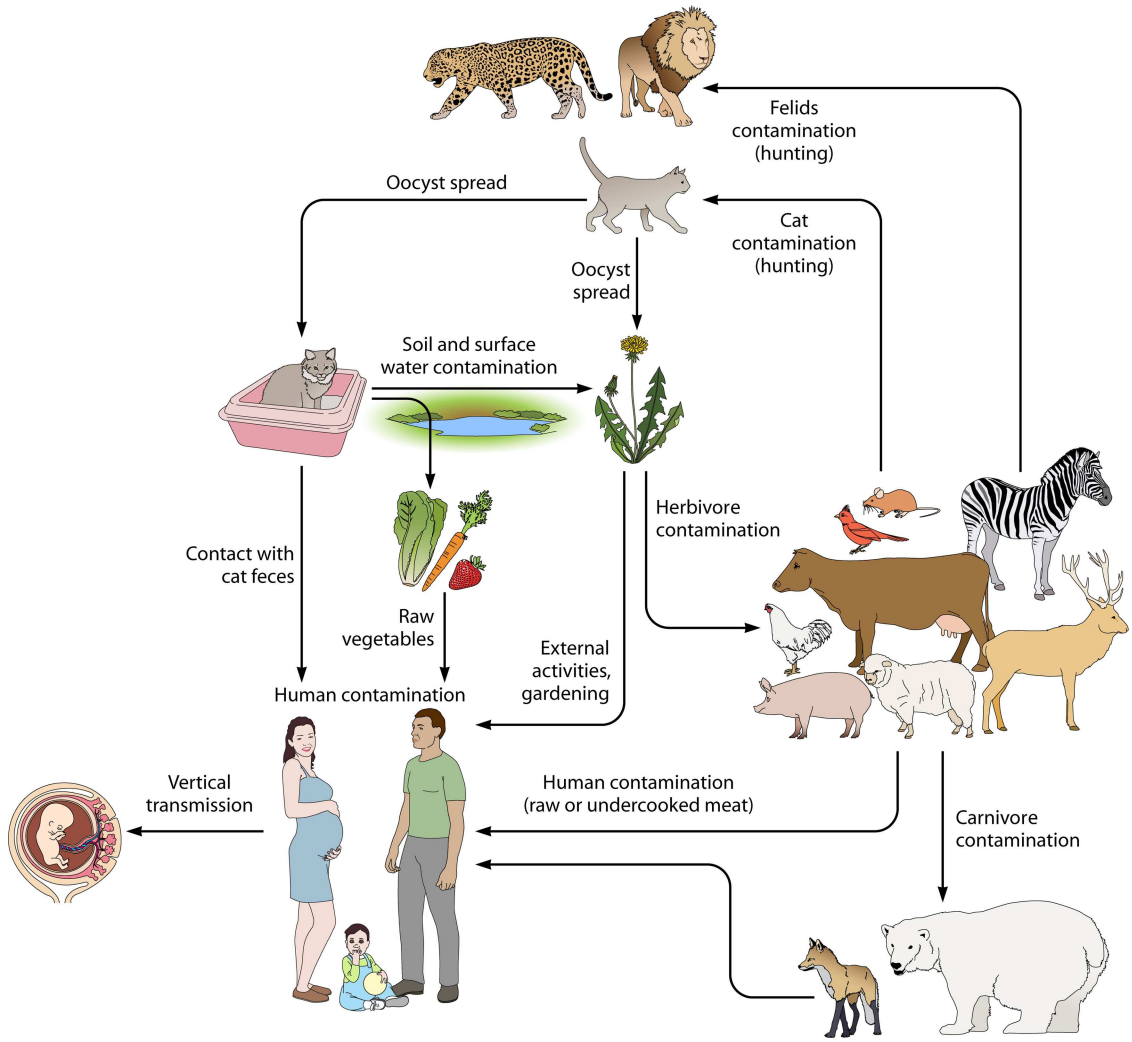

FIG 3 Sources of *T. gondii* infection in humans. The various sources of food-borne and environmental contamination of humans are represented.

ride or 1.4% potassium or sodium lactate are effective within 8 h of injection for the killing of *T. gondii* tissue cysts in pork loin (169).

Other food treatment processes, such as gamma irradiation at a dose of 1.0 kGy and high pressure (300 mPa), were found to be efficient for killing tissue cysts in meat, but some treatment procedures are barely applicable for meat destined for human consumption (206).

Infection related to solid-organ transplantation. As *T. gondii* tachyzoites can invade all nucleated cells, cysts can be found in virtually any organ. Therefore, in solid-organ transplantation (SOT), *Toxoplasma* infection can be transmitted through a cyst-containing organ from a donor (D) with infection acquired in the distant past to a nonimmunized recipient (R). However, certain organs are more likely to harbor persistent cysts than others. Muscles commonly sustain parasite encystment; thus, heart transplant patients are at a higher risk for organ-related toxoplasmosis than are liver, lung, or kidney transplant patients (Table 1). Toxoplasmosis was recognized early as an infectious complication in heart transplant patients (288), which motivated the implementation of large retrospective studies in several countries from 1980 onwards (Table 1). However, the incidence of acquired toxoplasmosis in case of a mismatch (D^+/R^-) is variable, since it depends largely on the prevalence of toxoplasmosis in the country of study and on the use of chemoprophylaxis after transplantation. In retrospective studies, the incidence can vary from 9 to 56% when the patients benefit or not from a chemoprophylaxis scheme, respectively, indicating that prevention is efficient (Table 1). In a recent multicenter retrospective study including 22 patients with acquired toxoplasmosis within a median time of 92 days posttransplantation, mismatched transplants were documented for 9 patients, and the donor's serology was unknown for 8 other negative recipients (125). Twelve of 22 cases were heart transplant patients. The incidence of donor-acquired toxoplasmosis is less frequent in other SOT patients, and only 9 and 16 cases were reported for liver- and kidney-mismatched patients, respectively, supported by solid serologic evidence (Table 1). A case of disseminated toxoplasmosis following small bowel transplantation was also described, but the serostatus of the donor was unknown, making the source of infection uncertain (48).

TABLE 1 Reported cases of organ-related acquired toxoplasmosis in solid-organ transplant patients, 1980 to 2011[j]

Yr/country of study	Type of study	Transplant	No. of patients	No. of mismatches/no. of cases with chemoprophylaxis	No. of cases of acquired toxoplasmosis	No. of *Toxoplasma*-related deaths	Reference(s)
1979–1988/United Kingdom	Retrospective	Heart	250	21/8	6	2	339
1980–1996/United States	Retrospective	Heart	575	32/16	4	4	229
1985–1993/United Kingdom	Retrospective	Heart	290	13/13	1[a]	0	244
1984–1997/Canada	Retrospective	Heart	205	20/20	0[b]	0	154
		Liver	186	15	1[c]	0	
		Kidney	516	39	2[d]	0	
1985–1991/Switzerland	Retrospective	Heart	121	18	14[e]	0	139
1986/United Kingdom	Retrospective	Heart	119	14	4	2	161
1988–2003/Spain	Retrospective	Heart	315	32/29	2	0	234
1989–2004/United States	Retrospective	Heart	596	UD/596	0	0	22
1987–2000/United States	Retrospective	Heart	377	45/45	4[f]	0	19
1989–2006/Italy	Retrospective	Heart	119	66/66	12[g]	0	294
1989–2008/Germany	Retrospective	Heart	344	30/30	UD[h]	0	88
2000–2009/Spain	Retrospective	Heart	1,979	9/UD (all organs)	12	3 (all organs)	125
		Liver	7,709		6		
		Kidney	4,872		4		
1983–2005/miscellaneous	Case reports	Heart	NA	14/UD	14	8	13, 135, 153, 165, 172, 211, 226, 236, 286, 293
1987–1997/Switzerland	Retrospective	Liver	98	UD	3	0	140
1983–1988/United Kingdom	Retrospective	Liver	40	UD	1 (mismatch)	0	292
1995–2007/miscellaneous	Case reports	Liver	NA	5	5	3	37, 39, 56, 219, 323
1983–2010	Case reports	Kidney	NA	16/2[i]	16	9	214, 217, 270, 282

[a] Four patients were lost to follow-up.
[b] Seven patients were lost to follow-up.
[c] Three patients were lost to follow-up.
[d] Twelve patients were lost to follow-up.
[e] Eleven asymptomatic cases.
[f] Three asymptomatic cases.
[g] Nine asymptomatic cases.
[h] There were 46 seroconversions in the whole population study.
[i] Six patients without chemoprophylaxis and 8 with unknown data.
[j] UD, unknown data; NA, not applicable.

Infection through Oocysts

Survival of oocysts in the environment. As highlighted by previous epidemiological studies, environmental conditions are important for oocyst survival. Moist conditions can increase oocyst survival during long periods of heat, which likely accounts for the high prevalences in tropical countries of South America and Africa. In Colombia, a correlation was found between the mean amount of rainfall and the incidence of congenital toxoplasmosis (152). Even in a country with a temperate climate, such as France, the risk of infection in cats was shown to increase when the weather was both warm and moist, or moderate and less moist, reflecting the influence of climatic conditions on the prey population and oocyst survival (3).

Despite the low prevalence (<1% in most studies) and short duration of oocyst shedding by cats, the burden in the environment may be very high (3, 66). A single cat may shed more than 100 million oocysts, which are nonsporulated (Fig. 2). These oocysts need between 1 and 5 days to mature and become infective for other hosts, which explains why direct contact with cats is not thought to be a major risk for human infection. In the United States, an increased risk associated with exposure to kittens was limited to respondents who had 3 or more kittens, thus more likely to be infected through the shedding of oocysts after primary infection (179). Oocysts are able to sporulate within 2 to 3 days in different types of commercial cat litter and occasionally remain viable for 14 days (94). Unsporulated oocysts lose their capacity to sporulate, and, hence, to become infective, after freezing at −6°C during 7 days or after exposure to 37°C for 1 day. Once sporulated, oocysts are resistant to harsh environmental conditions. They remain viable in a moist environment for more than a year. Under laboratory conditions, sporulated oocysts can survive storage at 4°C for up to 54 months. They survive freezing at −10°C for 106 days and heating at 35°C and 40°C for 32 days and 9 days, respectively. However, they are killed within 1 to 2 min by heating to 55°C to 60°C (93), conditions easily obtained when cooking vegetables. The wall of sporulated oocysts is highly impermeable and, therefore, very resistant to disinfectants (110).

Contamination of water. Oocysts can remain viable for long periods of time in water and can resist freezing and moderately high water temperatures. They are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment (111). Outbreaks associated with the contamination of reservoirs supplying water, such as those described for the Greater Victoria area of British Columbia, Canada (16); in Santa Isabel do Ivai, Brazil (76); or in

Coimbatore, India (21), involved a large number of patients. The epidemics were preceded by peaks of heavy rainfall and turbidity in the implicated reservoirs. Smaller epidemics were described after the drinking of raw surface water in remote tropical areas (29, 75). Freshwater runoff from urban centers next to seashores may contaminate seawater. *Toxoplasma* oocysts can remain viable for extended periods of time in seawater (207). Shellfish are filter feeders that concentrate *T. gondii.* Oocysts remained viable and were detected in various species of shellfish under natural conditions (119, 228, 263). The consumption of oysters, clams, and mussels has been shown to be a risk factor for acquiring *Toxoplasma* infection in the United States (179).

The detection of *Toxoplasma* in water is difficult, and no standardized methods are available. The methodology is based on the experience gained from other coccidians, such as *Cryptosporidium*, and involves the concentration of oocysts using centrifugation, filtration, immunomagnetic separation, or flocculation of large volumes of water (109, 110, 300). Different PCR methods have been proposed (308, 329). In France, a survey found *Toxoplasma* DNA in 7% of raw surface water samples. Well water was PCR positive in 9% of samples in this French study (329) and also in 13 to 27% of samples in Poland, depending on the depth of the well (309). A positive correlation was observed between the consumption of unboiled well water and the presence of *Toxoplasma* antibodies, especially for farms with poor-hygiene conditions surrounding shallow wells.

Contamination of soil, vegetables, and fruits. Contact with soil was identified as a strong risk factor in a European multicenter case-control study, and 6 to 17% of primary infections in humans were attributed to this risk factor (61). A U.S. study showed that the detection of antibodies against *Toxoplasma* was 2-fold higher in a population with positive *Toxocara* antibodies, suggesting a common exposure to contaminated soil (182). The risk of acquiring *Toxoplasma* infection after soil contact or ingestion is particularly high for children. *Toxoplasma* oocysts were isolated in as many as 32% of school playgrounds in a Brazilian study (89).

Contaminated water and soil may act as vehicles for the transfer of oocysts to vegetables and fruit for human consumption, although there are few data available to confirm this. In several risk factor or case-control studies, the eating of unwashed raw vegetables or fruits was associated with an increased risk of primary infection (31, 185, 210). Experimentally, *T. gondii* oocysts can adhere to berries, especially raspberries, and can be recovered by bioassays in mice (192), but there has been no report of the detection of *Toxoplasma* on fruits or vegetables under nonexperimental conditions.

Infection through Tachyzoites

Food-borne contamination. Outside its host cell, the tachyzoite is a fragile stage, easily destroyed by digestive enzymes (10-min survival in pepsin-HCl). It is also very sensitive to environmental conditions and is usually killed rapidly outside the host. Therefore, the horizontal transmission of *Toxoplasma* via tachyzoites is probably not important from an epidemiological point of view. However, tachyzoites were suggested to be the cause of rare cases of acquired toxoplasmosis in humans after the consumption of unpasteurized goat's milk (316). The drinking of unpasteurized goat's milk was found to be a risk factor in an epidemiological survey (179), suggesting that tachyzoites may enter the host by the penetration of mucosal tissue.

Congenital infection. When primary infection is acquired by a pregnant woman, tachyzoites can colonize placental tissues during the dissemination process and from there can gain access to the fetal compartment in about 30% of cases. The frequency of vertical transmission increases with the gestational age at maternal infection. At the beginning of pregnancy, the transplacental passage of tachyzoites is a rare event, but the consequences for the offspring are heavy. The immune control of placental infection is probably a key event in the occurrence of congenital infection (254), but advances in the comprehension of the pathophysiological process remain to be achieved.

Congenital infection is the most important part of the disease burden due to *Toxoplasma* infection in humans. Clinical manifestations of congenital toxoplasmosis first motivated research on the parasite and its pathophysiology and epidemiology. However, the factors influencing congenital transmission are still poorly known, apart from the term of pregnancy at the time of maternal infection and, of course, the immune status of the mother.

The observation of a decreasing seroprevalence of toxoplasmosis in industrialized countries has complex consequences for the risk of acquisition of *Toxoplasma* infection during pregnancy. At first glance, a reduced seroprevalence increases the percentage of pregnant women susceptible to primary infection and, hence, to congenital transmission to their fetuses. However, the lower level of circulation of the parasite in the environment diminishes the global risk of acquiring infection during pregnancy. A national surveillance system was implemented in France in 2007, which aims to collect data from all cases of congenital toxoplasmosis through data transmitted by laboratories certified for prenatal diagnosis or implicated in neonatal serological diagnoses. This network reported 272 cases of congenital toxoplasmosis in 2007 (328). If these data can be considered exhaustive, they can allow an evaluation of the overall prevalence of congenital toxoplasmosis in France, 3.3 per 10,000 live births (328), which is nearly the prevalence reported in Brazil (1 per 3,000 live births) (238) but 3-fold higher than that estimated in a pilot study in Massachusetts (1 per 10,000 live births) (159).

Transmission through injection. Fourteen cases of laboratory contamination of a parenteral origin have been described (166). Most cases were attributed to needlestick injuries or scratching while manipulating tachyzoites from the RH strain.

The risk of transmitting infection through a blood transfusion is theoretically possible if the donor has recently acquired a *Toxoplasma* infection and is parasitemic at the time of blood sampling. Similarly, a risk associated with bone marrow is possible if the donor is parasitemic at the time of collection. However, the maximal duration of dissemination of tachyzoites through the blood flow is barely known for humans; it may depend on the parasite strain and on the host immune response. Parasite DNA was detected in 9 out of 17 patients during 5 weeks following acute toxoplasmosis with lymphadenopathy (160). In a mouse model, it was also shown by PCR that parasitemia was detected during 3 weeks after oral infection (249).

POPULATION STRUCTURE OF *T. GONDII*

Genotypes and Their Geographic Distribution

Studies of genotypes of *T. gondii* began in the early 1990s and at first relied on isoenzyme analysis (69, 70) and on a few PCR-

TABLE 2 Geographical distribution of *Toxoplasma gondii* genotypes and possible relationships with human disease

Geographical area	Genotypes	Specific features of human disease in immunocompetent individuals and those with congenital toxoplasmosis
Europe	Type II (haplogroup 2), highly predominant; type III, more present in South Europe; other genotypes sporadically observed	Asymptomatic or benign disease in immunocompetent individuals associated with type II or III; lower rate of retinochoroiditis in immunocompetent patients and in those with congenital toxoplasmosis than in areas of South America
North America	Type II (haplogroup 2), haplogroup 12, type III (haplogroup 3), other genotypes	Asymptomatic or benign disease in immunocompetent individuals associated with type II or III; not enough data for other haplogroups
South and Central America	High genotypic diversity; some haplogroups shared with Africa (haplogroup 6); type II sporadically present; type I rarely encountered; highly atypical genotypes in the Amazonian forest	Higher rate and severity of retinochoroiditis in immunocompetent patients and in those with congenital toxoplasmosis; disseminated, potentially lethal, cases observed with the most atypical genotypes
Africa	African 1, 2, 3 (haplogroup 6); type III (haplogroup 3); type II	Data showed a higher rate of retinochoroiditis than in Europe
Asia	Less genotypic diversity than in South America; type III (haplogroup 3); a common haplogroup widespread across the continent	No comparative data

restriction fragment length polymorphism (RFLP) markers (175). Genotyping was later refined by the addition of new PCR-RFLP markers (313) and by microsatellite analysis (10, 11). The sequencing of selectively neutral introns was proposed to be a better tool for phylogenetic studies (187), whereas microsatellites are better adapted to population genetic structure and outbreak investigations (75, 223, 337).

Despite the presence of a sexual cycle and a worldwide distribution, the population structure of this parasite was initially described as being highly clonal and exhibiting a low genetic diversity. This was the conclusion of genetic studies of isolates from Europe and the United States, which grouped these isolates into three major multilocus genotypes, types I, II, and III, equivalent to clonal lineages, stable in time and space (8, 69, 70, 175, 303). This simple clonal structure is accompanied by a low level of genetic divergence among the three lineages (only ~1 to 2% divergence at the DNA sequence level between lineages). However, multilocus and multichromosome genotyping of isolates from other continents revealed a much more complex population structure with a greater genetic diversity, likely reflecting a history of more frequent genetic exchanges and genetic drift (7, 203) (Table 2). The majority of isolates from South America, Africa, or Asia do not fit into the three major lineages (with the exception of type III, which is really cosmopolitan). The clustering of these genotypes led to the description of new haplogroups, some of them largely distributed over continents, being considered other successful clonal lineages (187, 223, 251). Ongoing efforts are aimed at gathering data from analyses with different markers (PCR-RFLP, microsatellites, and sequencing of introns) to establish a consensus nomenclature for these haplogroups, which may be useful for basic biology as well as for clinical studies. Up to now, 12 haplogroups (including the 3 initially described lineages, types I, II, and III) have been described (186, 187), based on sequence-based analyses, but these haplogroups are not totally homogenous, and more resolutive markers revealed subclustering that may be associated with geographical origins and phenotypic characteristics. There still remain truly atypical and highly diverse isolates with many unique polymorphisms which cannot be clustered into one of these haplogroups (222).

From Northern Europe (178) to Southern Europe (86), the population structure of *T. gondii* is markedly clonal, with a predominance of strains belonging to the type II lineage. In France, type II strains represent more than 90% of isolates from both humans and animals (9, 18, 162). Two other clonal lineages are occasionally (type III) or exceptionally (type I) found in Europe. Type III may be more frequently encountered in Southern Europe (86, 331). The isolation of atypical strains which do not fit into these 3 major lineages is rare in Europe and likely suggests contamination by non-European strains either during residence abroad or after the consumption of imported food (12, 261). In North America, the population structure appeared similar to that observed in Europe, with a predominance of type II strains (175), but recent data suggest a higher prevalence of atypical strains in North America in wild as well as in domestic animals (103, 106) and another clonal haplogroup (haplogroup 12) close to type II (186). South America is an area with a high level of diversity for *T. gondii*. Although additional clonal lineages, known as the Br I to IV haplogroups, may be common and endemic in Brazil, it is clear that frequent genetic exchanges have generated a wide variety of different genotypes (7, 251). Eighty-eight genotypes (defined with 11 PCR-RFLP markers) have already been identified from a variety of animal hosts in Brazil, and new genotypes are continuously being identified in different animal species, indicating an extremely high level of diversity of *T. gondii* in the population (252), whereas type II seems to be very rare in South America (102). The high level of genetic diversity observed in this continent is maximal in the wild Amazonian area, with many unique polymorphisms (7). In an Amazonian country such as French Guiana, the interpenetration of anthropized and wild rainforest environments leads to hybridization between strains that may represent a potential risk for human health (222). In Africa, a clonal population structure consisting of additional common clonal lineages known as the Africa 1 to 3 haplogroups, coexisting with type II and III lineages, has been described (12, 223, 326). In Asia, the first reports from China, Sri Lanka, and Vietnam (98, 104, 341) revealed a more limited genetic diversity than in South America, with some genotypes being common to both areas. In China, a clonal lineage

seems to be widespread across the country (341). The distribution of the different genotypes is summarized in Table 2.

Hypothesis on the Origin of Genotype Distribution

The three major lineages in Europe and North America are thought to have resulted from a few natural genetic crosses between highly similar parental types, the progeny of which expanded to give rise to the clonal population structure during the past ~10,000 years (43, 312). The clonal expansion of these three lineages may have been facilitated by an enhanced fitness, being able to effectively outcompete other genotypes (302). The domestication of cats, farming with the subsequent proliferation of domestic rodents, and the breeding of a narrow range of domestic animals, which began in the Middle East and the Mediterranean area ~10,000 years ago, may have favored this expansion. The same phenomenon of outcrossing followed by the expansion of clonal lineages may be applied to the additional clonal lineages on other continents. The geographical expansion of identical genotypes across continents or between continents may also have been facilitated by human activities, such as transportation and human exchanges (223), but also by the migration of birds (262). Recent data showed that African and American strains share an ancestor, supporting the hypothesis of *T. gondii* migration on ships together with cats and rodents during the transatlantic slave trade during the 18th and 19th centuries (223). The influence of human activities on the population structure of *Toxoplasma* could also be found in the lower level of genetic diversity of the parasite found in the anthropized environment than in the wild environment (7, 222).

South American isolates have diverged from North American isolates. By calculating the extent of the geographical allelic diversity, it was estimated that this split occurred approximately 10^6 years ago (187). This corresponds to the same time frame as the reconnection of the Panamanian land bridge, allowing *T. gondii* migration into South America with felids (187). However, the worldwide evolutionary history of *T. gondii*, on different time scales, will need more samplings from different continents, and notably in Asia, the birthplace of the felids.

Genotypes and Virulence

Experimental virulence is usually defined with the mouse model after the intraperitoneal inoculation of a given number of tachyzoites. Type I isolates are highly virulent, leading to the death of mice less than 10 days after the inoculation of <10 tachyzoites, while type II or III strains are considered avirulent strains, allowing survival after the inoculation of $>10^3$ tachyzoites. Isolates from other clonal lineages or from atypical strains range from the highly virulent to the intermediate or nonvirulent phenotype, according to differences in the combination of genes that they have inherited (68, 158, 289). Genotypes with a majority of type I alleles are usually more virulent (223).

The mouse-virulent strains display several characteristics that may explain the rapid dissemination of the parasite and the higher tissue burden observed for mice and other susceptible hosts: enhanced migration across polarized epithelia or across the extracellular matrix, higher rates of the *ex vivo* penetration of the lamina propria and submucosa (25), and, in cell culture, higher growth rates and lower rates of interconversion from tachyzoites to bradyzoites (289). Experimental crosses between strains with different virulence patterns facilitated the identification of several polymorphic genes coding for secreted factors of *Toxoplasma* associated with differences in the expression of virulence in mice (266, 290, 315). These key virulence factors are secretory proteins discharged from apical organelles, the rhoptries. The proteins of this rhoptry family (ROP5, ROP16, and ROP18) exert kinase or pseudokinase activity. They are injected directly into the host cell and play a role during the process of parasite invasion or in the induction of interleukin-12 (IL-12) secretion by mouse macrophages (272).

Although these biological and genetic data demonstrate the different intrinsic properties of the different strains, the expression of this virulence in a given host species is a more complex trait which depends on several host and parasite characteristics. Different host species are more or less susceptible. The genetic background of a given species, as demonstrated for different mouse or rat strains, may also influence the expression of virulence (51).

The expression of virulence in humans is a complex phenomenon due to many other factors that could influence the pathogenicity of a given strain: other parasitic factors (infectious stage and inoculum), the genetic background of the host, and overall immune status (218). *Toxoplasma gondii* is usually considered an opportunistic parasite in humans, and any analysis of the relationship between genotype and pathogenicity should consider these different factors. This explains why the role of the strain is still a matter of debate, especially when the host is immunocompromised. Strains isolated from patients are mainly the strains circulating in a given country, and the same type of strain can be responsible for different outcomes. For example, type II strains were involved in 96% of consecutive cases of congenital toxoplasmosis in France (9), in 85% of immunocompromised patients who acquired *Toxoplasma* infection in Europe (12), and in 73% of cases of ocular disease in France (121). In immunocompromised patients, the conclusion of a study of 85 patients (HIV and non-HIV immunodeficient patients) was that the genotype of the infecting strain had no influence on the clinical manifestation (cerebral or extracerebral) or clinical outcome (12), indicating that immune status is responsible for virulence expression in these patients.

However, several direct and indirect arguments plead for an influence of the strain on clinical severity. In immunocompetent patients, severe toxoplasmoses with multiorgan failure were linked to atypical strains acquired from the Amazonian rainforest (49). Occasional reports of such severe cases due to atypical strains have come from other countries (82), sometimes after the consumption of infected food (261). The high rate of occurrence of acquired ocular toxoplasmosis in Southern Brazil (21% in individuals over 13 years of age) has been attributed to the genotypes circulating in this region (73, 188). In cases of congenital toxoplasmosis, the strain was likely to play a role in the different outcomes observed by a comparative prospective cohort study of congenitally infected children in Brazil and Europe (146). In France, where systematic diagnoses of congenital toxoplasmosis were performed, type II isolates were found in all different aspects of congenital disease, from lethal infection to latent toxoplasmosis, classically depending on the term of pregnancy during which the infection was acquired. On the other hand, the few atypical isolates detected in this country were observed only for severe cases of congenital toxoplasmosis (9, 74). The possibility of reinfection by a different strain is another consequence of this genetic diversity, raising the new concept that immunity against one strain may not be completely protective against another one, as shown for a case

of reinfection with an atypical strain leading to severe congenital toxoplasmosis (115).

CLINICAL FEATURES OF TOXOPLASMOSIS IN HUMANS

Pathogeny and Development of the Immune Response during the Course of Infection

Following the ingestion of cysts or oocysts, the respective excysted forms, bradyzoites or sporozoites, rapidly invade the small intestinal epithelium, where they convert into tachyzoites. The acute early steps of intestinal infection of humans are not well characterized, but the establishment of infection probably relies on the intrinsic properties of the parasites. First, the high motility of tachyzoites and cell interactions between the parasite protein MIC2 and the host intercellular adhesion molecules (ICAM-1) could be used for paracellular crossing. Moreover, the active invasion of the apical side of the epithelial cell could be followed by egress from the basolateral side (transcellular traversal) (198). Whatever the early scenario comprising or not an initial multiplication of tachyzoites in the intestinal epithelium, they further cross the intestinal barrier and invade monocyte cells in contact with the lamina propria, which are key cells for the dissemination of *Toxoplasma* through the blood flow toward all organs, using them as Trojan horses to cross biological barriers (24, 35), as shown with a murine model of infection by intracellular fluorescent parasites (322). This peculiar capacity to actively invade all nucleated cells, including professional phagocytic cells, contributes to the complexity of the host-parasite interactions through the direct modulation of the host immune response.

The cellular and soluble effectors involved in the immune response against *T. gondii* have been extensively studied in the last 2 decades. It was recognized early that a T helper 1 (Th-1) immune response driven by gamma interferon (IFN-γ)- and interleukin-12 (IL-12)-producing cells is essential for the control of the parasite burden. The fine regulation of immune effectors and their signaling pathways were reviewed recently by Miller et al. (227). Briefly, following the ingestion and transepithelial transfer of parasites, there is a local release of chemokines by infected cells, leading to the attraction of cells of the innate immunity. Neutrophils are attracted to the infected foci early to phagocytose free parasites and contribute to reducing parasite burdens (38). Other phagocytic cells, such as dendritic cells (DCs) and macrophages, play a pivotal role in the initiation of innate immunity, as they are the major sources of IL-12 as well as IL-18, thus promoting natural killer (NK) and NKT cell activation (177), with both cell types producing IFN-γ in large quantities (132). Moreover, DCs and macrophage cells can present parasite antigens associated with major histocompatibility complex (MHC) class II antigens and costimulatory molecules and further prime T cells (59). In addition, DCs and NK cells can also interact directly, with this dialog resulting in the mutual activation and amplification of IL-12 and IFN-γ synthesis, respectively. Classically, the release of IFN-γ can trigger macrophage activation to synthesize tumor necrosis factor alpha (TNF-α), thus being responsible for an amplification loop. The further recognition of parasite antigens by pattern recognition receptors (PRRs) leads to an exacerbation of phagocytic activity with an enhanced production of reactive oxygen species (ROS) and nitric oxide (NO) species and tryptophan starvation through 2-3-indole-amine dioxygenase (IDO) activation (255).

However, this potent machinery has two limitations. The first limitation resides in the negative counterpart of a strong Th-1 immune response, which may overwhelm its goal and be responsible for severe inflammation, resulting in intestinal tissue damage or even the death of the susceptible host, as shown with a murine C57BL/6 model (204). Thus, there is a need for downregulating effectors, a role devoted at least partially to IL-10 and transforming growth factor β (TGF-β), which modulate macrophage activation (233). Such a deleterious effect of an acute Th-1 immune response is also well known in the setting of primary acquired infection during pregnancy and can result in fetal loss, since IFN-γ destabilizes the Th-2 microenvironment necessary for maternal-fetal tolerance. Thus, the complexity of the maternal-fetal interface is magnified by *Toxoplasma* infection, and the role of the placenta in the immunomodulation process is probably essential for the maintenance of gestation after maternal infection (254, 276).

On the other hand, despite the powerful host cell effectors described above, recent data provided mechanistic details on how *Toxoplasma* surrounds the host immune system, thus making itself a successful parasite persisting lifelong in host tissues. It is now recognized that the parasite rhoptry protein ROP16 can rapidly process into the host cell nucleus, where it interferes with signaling pathways of host immune responses, particularly through the phosphorylation of the STAT3 and STAT6 transcription factors (197, 291), leading to the downregulation of IL-12 production by macrophages and, subsequently, of IFN-γ (77). Interestingly, this capacity is not shared by all strains but is devoted to type I and III isolates (290, 291). This could partially explain the greater severity usually observed for infections due to strains harboring type I alleles. Moreover, type II strains, which do not exert this capacity to repress the host response, induce a rapid immune response, limiting parasite growth, thereby ensuring the survival of both the host and parasite and resulting in bradyzoite conversion and the encystment of the parasite for persistence. At the same time, it was shown that *Toxoplasma* can also inhibit apoptotic mechanisms of the infected cell by antagonizing caspase 8 (330) and interfering with the NF-κB pathway (197), thus ensuring both protection against the rapid clearance of intracellular tachyzoites by macrophages and the long-term survival of bradyzoites in the cysts.

In the immunocompetent host, the occasional rupture of individual cysts is considered to be responsible for the continuous stimulation of the immune response, which ensures a dynamic control of the cysts.

Toxoplasmosis in Immunocompetent Subjects

Primary acquired infection is asymptomatic in more than 80% of cases of immunocompetent subjects in European countries or North America (230). In the remaining cases, patients may experience fever or cervical lymphadenopathy, sometimes associated with myalgia, asthenia, or other nonspecific clinical signs. Lymphadenopathy and asthenia may persist for several weeks, mimicking infectious mononucleosis, especially since monocytosis can be observed on blood smears. A study conducted in the United States showed that only 48% of mothers who gave birth to congenitally infected infants could recall clinical signs suitable with toxoplasmosis during their pregnancy (42). More rarely but not exceptionally, toxoplasmic chorioretinitis with visual impairment may reveal primary infection (72, 232), although it was previously thought that ocular toxoplasmosis was the result of congenital infection. Indeed, in a retrospective study by Delair et al., 100 out

of 425 (23.5%) consecutive cases of ocular toxoplasmosis were attributed to acquired toxoplasmosis (72).

In fact, it is now recognized that the severity of infection may depend on the genotype of the strain. Indeed, as stated above, the severity of infection is low in Western European countries and North America, where type II strains predominate (175), but much higher in other parts of the world, such as South America (49, 75) or Africa, where other genotypes circulate (188, 223, 324). In particular, several studies have shown higher incidences and severities of chorioretinitis in Brazil (146) or Colombia, both during primary infections of immunocompetent subjects and in congenitally infected infants. The strain genotype could also be an explanation for the high proportion of retinochoroiditis (19 of 100 cases with proven acute infection) in an outbreak in Victoria, British Columbia, Canada, where an atypical cougar isolate was suspected to be the cause (40), and for the 100-fold-higher incidence of ocular toxoplasmosis in patients born in Africa than in patients born in Britain (148). Moreover, in the British Columbia outbreak, 20% of patients had recurrent episodes of retinochoroiditis (47).

Such strains with atypical genotypes can also be responsible for severe or lethal infections in immunocompetent subjects, which may take the form of pneumonitis, myocarditis, meningoencephalitis, or polymyositis. Data collected through the Resource Biological Center of the Centre National de Référence de la Toxoplasmose (Limoges, France) showed that among the few severe or lethal infections that occurred from 2007 to 2010 in France, 7 of 10 were related to atypical genotypes acquired in French Guiana (49).

Toxoplasmosis in Immunocompromised Patients

Contrasting with the setting of *Toxoplasma* infection in immunocompetent subjects, toxoplasmosis is always life threatening in immunocompromised patients, whatever the strain, yet the host immune background is of prime importance. Various factors responsible for profoundly impaired cellular immunity can lead to severe toxoplasmosis, among which are HIV infection and immunosuppressive therapies. Patients are more commonly at risk for disease reactivation resulting from cyst rupture than for a newly acquired infection, but the risk may differ among categories of patients. In transplant patients, severe or disseminated toxoplasmosis can result from either a reactivation of latent infection in the recipient or infection from a cyst-containing organ from a seropositive donor given to a seronegative recipient (39, 214, 282), a situation where heart transplants carry the highest risk (139, 293). A reactivation of a chronic infection may occur in the recipient irrespective of the type of graft, but the risk is closely related to the duration and degree of immunosuppression, with hematopoietic stem cell transplant (HSCT) patients being most at risk (78, 81, 281). In HIV-infected patients, the incidence of toxoplasmosis is closely related to $CD4^+$ T cell counts, with an increasing risk when the count falls under 100 cells/μl. Toxoplasmic encephalitis (TE) is the most predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache, lethargy, incoordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizures, usually associated with fever (212). The incidence of TE has decreased since the use of highly active antiretroviral therapy (HAART) (183), as was shown with a French cohort, where the risk was divided by 4 and fell from 3.9 to 1.0 cases per 100 person-years (2). Other organs can be involved, either because they are target organs for encystment and thus are subsequent potential sites for cyst reactivation or because they are secondarily infected following the dissemination of parasites from an initial reactivation site. After the brain, the most frequently involved organs are the lungs, the eyes (265), and the heart, resulting in myocarditis, but the isolation of *Toxoplasma* from many other sites, such as liver, pancreas (171), bone marrow, bladder (264), lymph nodes, kidney, spleen, and skin (17), has been documented. Pulmonary or disseminated toxoplasmosis is seen mostly in transplant patients, who develop rapidly progressive infection and a massive dissemination of parasites (39, 78, 248).

Less frequently, toxoplasmic retinochoroiditis may occur independently of any other signs of evolutive infection and must be distinguished from other infectious etiologies, in particular eye lesions due to cytomegalovirus (CMV), HIV, or syphilis.

Congenital Toxoplasmosis

Classically, congenital infection results from primary acquired maternal infection during gestation. The frequency of vertical transmission and the severity of fetal damage depend on the stage of pregnancy when maternal infection occurs. The placenta plays a main role in the process, as it is both a natural barrier which is supposed to protect the fetus and a target tissue for parasite multiplication (1). In fact, the placental barrier is more efficient at the beginning of gestation, leading to the passage of parasites in less than 10% of cases during the first trimester, but becomes more permeable throughout pregnancy, allowing parasite transmission in around 30% of cases in the second trimester and 60 to 70% of cases in the third trimester and even more close to the time of delivery (113). The severity of fetal infection is inversely correlated, since neonates are asymptomatic in more than 80% of cases when infected during the third trimester of gestation (83). However, when transplacental transmission occurs during the first trimester, the consequences for fetal development are heavy, often leading to severe abnormalities or to abortion. Parasite multiplication induces necrosis foci and strong inflammation, leading to major abnormalities in the brain and eye tissues. It can induce the destruction or profound remodeling of the white substance. Infected necrotized foci may block the aqueduct of Sylvius, resulting in hydrocephalus of lateral ventricles. These foci further calcify and can be detected by transfontanellar echography or cranial X ray. Major sequelae include mental retardation, seizures, microcephalus, hydrocephalus, deafness, and psychomotor deficiency (269). Eye lesions are also more severe in early pregnancy, where microphthalmia, cataract, increased intraocular pressure, strabismus, optic neuritis, and retinal necrosis can be observed (71, 280), as can uveitis and retinochoroiditis, possibly leading to blindness if retinal lesions affect the macula. During the second trimester, fetal infection can be of variable severity. Echographical ultrasounds may reveal areas of hyperechogenic mesentery, hepatosplenomegaly, or cerebral calcifications. Clinical manifestations at birth may include epilepsy, anemia, thrombocytopenia-induced petechiae, rash, hepatic disorders, pneumonitis, or retinochoroiditis (269). In a prospective European study, intracranial lesions detected at birth were associated with serious neurologic disorders in about 30% of cases (64). Among the data from 272 cases collected in 2007 through the French Surveillance Network (328), 11 cases resulted in the termination of pregnancy owing to cerebral lesions or fetal death, and 87% of live-born infants were asymptomatic. The remaining 13% of cases had intracranial calcifica-

tions (14 cases), hydrocephalus (3 cases), and/or retinochoroiditis of variable severity (12 cases).

Retinochoroiditis is a common feature that can be observed whatever the time of maternal infection. Its particularity resides in its frequently delayed clinical expression after birth. During a longitudinal U.S. study including 25 infants who were not treated *in utero* or during their first year of life, Phan et al. (256) observed that 72% of these infants developed new eye lesions during a mean follow-up time of 5.7 years. Another prospective study of 102 infants who benefited from antenatal and postnatal treatment showed that 78% were asymptomatic during a median follow-up time of 7.8 years (32). A recent European cohort study showed that the risk of developing eye lesions by 4 years of age was highest for children with serious neurologic sequelae at birth but also significantly increased for those with intracranial lesions or hepatosplenomegaly (131). Conversely, children without retinochoroiditis detected by 4 months were at a low risk of developing eye manifestations by 4 years of age. In any event, the question of long-term pathogenicity may differ according to prevention protocols, and probably according to the strain genotype, as retinal lesions are more extensive in congenitally infected Brazilian infants (325). Indeed, a comparative prospective cohort study of congenitally infected children in Brazil and Europe showed that, independently of treatment, Brazilian children had a 5-times-higher risk than European children for developing eye lesions, and their lesions were larger, more multiple, more recurrent, and more likely to impair vision (146).

Although the vast majority of congenital infections results from primary acquired infection during pregnancy, parasite transmission can occur in rare instances in immunocompetent, previously immunized women who are reinfected with *Toxoplasma* during gestation (115, 144, 164, 193). A recent case benefited from genotyping, which revealed that reinfection was due to an atypical strain which was responsible for severe congenital toxoplasmosis, raising the temptation to take primary prevention measures even in previously immunized pregnant women, particularly in cases of travel to areas where atypical genotypes circulate (115). A reactivation of past infection in HIV-infected women can also lead to congenital transmission, as shown by several case reports (208).

Other exceptional cases of vertical transmission following maternal infection in the 2 months before conception have been described (84, 216), but in most cases, the immune background of the mother could explain the prolonged dissemination of the parasite and thus further placental colonization and transmission. Another concept which has emerged from the French experience of the systematic screening of pregnancies for >20 years is that parasite transmission can be delayed, since few asymptomatic neonates born to mothers with periconceptional infection were diagnosed with congenital infection after birth despite negative prenatal screening results (278). Indirect arguments also support this hypothesis, since in a study by Romand et al. (284), the sensitivity of prenatal diagnosis was lower in early pregnancy than in mid-pregnancy, suggesting that vertical transmission may be delayed for some women infected in early pregnancy (319). Thus, in rare instances, parasites could persist in the placenta and proceed into the fetal compartment only at the end of gestation, which could explain why neonates may not have any clinical or radiological signs *in utero* and at birth. However, such an occurrence is probably rare, and its global frequency is not easily appreciated.

STRATEGIES FOR DIAGNOSIS OF TOXOPLASMOSIS IN HUMANS ACCORDING TO THE IMMUNE BACKGROUND OF THE PATIENT AND THE CLINICAL SETTING

Depending on the patient's immune background and on the disease setting or clinical signs, various techniques can be performed to achieve diagnosis, either indirectly, by detecting antibodies of different isotypes, or directly, by detecting parasites or DNA (Table 3). Many techniques are available, which all have strengths and shortcomings, and data obtained by use of these techniques should be interpreted with critical knowledge. Parasite detection or isolation is far less common in immunocompetent patients, except for severe cases of acquired toxoplasmosis with multiorgan failure, where the parasite may be found in the blood or other pathological products several weeks and up to 2 months after contamination (49, 84).

Diagnosis in Immunocompetent Subjects

Clinical indications of serologic tests. The diagnosis of *Toxoplasma* infection in immunocompetent subjects relies on serology. Because infection is often asymptomatic, serologic diagnosis is usually retrospective and is used to determine the immune status in some situations, such as (i) in a pregnant woman, preferably at an early stage of pregnancy; (ii) in a patient with uveitis or retinochoroiditis with no known history of congenital infection; or (iii) in a graft setting, i.e., in organ donors or transplant recipients. Moreover, in patients with fever or lymphadenopathy, serologic testing is a main diagnostic tool, since it allows the differential diagnosis of CMV, Epstein-Barr virus, or HIV infections but also other diseases associated with lymphadenopathy, whether infectious or not (hematological malignancies).

Kinetics of the antibody response. Immunoglobulin A and IgM antibodies are produced during the first week following infection and reach a plateau within 1 month (Fig. 4). Specific IgE antibodies are also produced early and rapidly disappear (126). Levels of specific IgM antibodies usually decrease after 1 to 6 months; they become negative in 25% of patients within less than 7 months (155) but commonly remain detectable for a year or longer. Exceptionally, IgM can disappear within 3 months or be hardly detected. On the other hand, specific IgA was initially thought to have a shorter duration, but it was later shown that it could be detected until 9 months; thus, it cannot be a surrogate marker of recent infection (237). The precocity of specific IgG detection depends on the technique used. Indeed, serologic techniques using membrane antigens or whole parasites, e.g., a dye test, an immunofluorescence assay, or an agglutination test, can detect early the antibody response, which is first directed against parasite surface antigens. ELISA techniques, which use mainly mixtures of cytosolic or metabolic and surface antigens and which differ among manufacturers, detect IgG later. Variations both in the individual immune response and in the characteristics of the technique used affect the kinetics of IgG detection, the synthesis of which may be detected 1 to 3 weeks after the initial rise in IgM levels. Whatever the technique, IgG synthesis reaches a plateau within 2 or 3 months and then decreases more or less rapidly and persists lifelong at residual titers, which are highly variable among patients.

Serologic techniques and interpretation. (i) A wide range of techniques. Serologic assays have improved and diversified over the years (336). The Sabin-Feldman dye test, which is based on parasite lysis by serum antibodies in the presence of complement,

TABLE 3 Diagnostic strategies for toxoplasmosis according to patient and disease setting

Patient	Disease setting	Diagnostic approach	Technique(s)	Sample(s)
Immunocompetent patient, transplant recipient, or pregnant woman	Primary infection or determination of immune status	Serology	Routine, IgG/IgM detection[a]; complementary,[b] IgG avidity,[c] IgA detection, dye test,[d] Western blotting,[d] and ISAGA[e]	Serum
Fetus	Maternal primary infection	Prenatal diagnosis[f] based on parasite detection	PCR, mouse assay[b]	Amniotic fluid
Newborn	Maternal primary infection	Parasite detection	PCR, mouse assay[b]	Placenta, cord blood
		Serology	IgG/IgM[e]/IgA detection[b]	Cord blood serum and/or newborn serum
			Comparative Western blotting[b]	Neonate and mother sera in parallel
Immunocompromised patient	Cerebral or disseminated toxoplasmosis	Parasite detection[b]	PCR	Blood
			PCR, cell culture, mouse assay, and histology	CSF, BAL, tissue specimens
Immunocompetent or immunocompromised patient	Retinochoroiditis	Serology	Comparative Western blotting,[b] Goldman-Witmer coefficient[b]	Aqueous humor and serum in parallel
		Parasite detection	PCR	Aqueous humor

[a] Routine diagnosis relies mostly on ELISAs.
[b] Should be reserved for reference laboratories.
[c] For dating infection if IgM is detected, particularly in pregnant women or organ donors.
[d] When a confirmation of low IgG titers is needed.
[e] The immunosorbent agglutination assay is a reference technique to confirm IgM specificity and to detect IgM in congenitally infected neonates.
[f] Ministerial agreement is required in some countries.

has been the gold standard for many years in terms of sensitivity and specificity but is now performed by very few laboratories. Many methods have been developed, from indirect fluorescence antibody tests (IFATs) (267) to hemagglutination; enzyme-linked immunosorbent assays (ELISAs); capture ELISAs allowing the detection of specific isotypes IgM, IgA, or IgE; and immunosorbent agglutination assays (ISAGAs), also suitable for IgM, IgA, or IgE detection. Nowadays, most clinical laboratories use an ELISA for the routine screening of specific IgG and IgM, whereas other techniques are mostly reserved for reference laboratories. However,

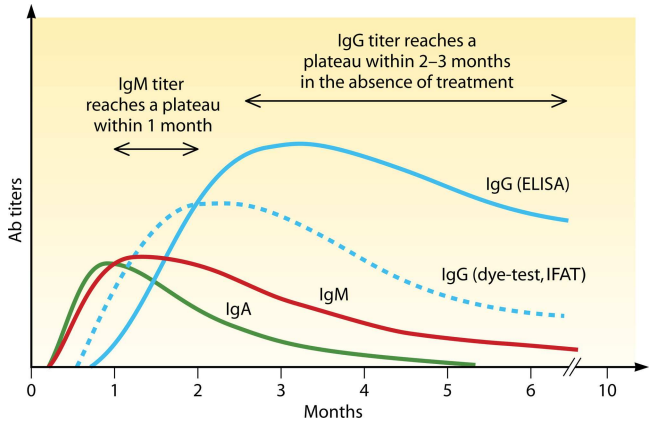

FIG 4 Kinetics of the antibody (Ab) response. The average kinetics of the different isotypes are represented, but they may differ among patients and according to the serologic technique used. IgM can be detected for years after infection.

the different methods on the market offer various performances and provide IgG titer results that are not comparable between each other, although they should be calibrated by comparison to an international standard. Therefore, the levels of IgG must be appreciated with the knowledge of the individual performance of the assay, and additional information on antibody kinetics must often be acquired from a second sample obtained 2 or 3 weeks later. Although most methods satisfy technical quality criteria for routine diagnosis, some situations require more specialized techniques for precise interpretations of the results.

(ii) The problem of low-level IgG detection. An everyday situation consists of correctly interpreting IgG ELISA results from the "gray zone," i.e., detecting low levels of IgG. In most cases, these low titers indeed correspond to specific IgG, but it must be clearly stated, particularly for organ donors or recipients, who could benefit from chemoprophylaxis. These low titers must be confirmed by using a dye test or a sensitive Western blot (WB) assay. A commercial Western blot assay available in Europe but not registered in the United States (WB Toxo GII; LDBio), which reveals specific IgG responses to several *T. gondii* antigens, including the tachyzoite major surface protein SAG-1, was shown to have a specificity of 100% and a sensitivity of 99.2% compared to the dye test (129).

(iii) The pitfall of IgM detection, or how to date the infection. One other common pitfall in serologic interpretations concerns IgM detection, the specificity of which must be confirmed by a second technique. Even if most techniques have gained in specificity, they also gained in sensitivity, which is a big concern, since most ELISA or ISAGA techniques can detect IgM for months or

years after infection. By monitoring a cohort of 446 women who acquired toxoplasmosis during pregnancy, Gras et al. (155) showed that IgM detection using ISAGA and IFAT persisted beyond 2 years in 27% and 9% of women, respectively. Therefore, IgM detection is no longer a marker of recent infection, unless it is found at high titers. Thus, a current means of confirming or ruling out a recent infection is the determination of IgG avidity (200), a method which relies on the progressive increase of the affinity of the antibody for its target antigen during the course of natural immunity following infection. The measure of the strength of antibody binding can be evaluated by an ELISA by introducing a washing step using a dissociating buffer (usually urea), which can remove the low-avidity antibody from a recently acquired infection. The resulting titer of detectable IgG is used to calculate a ratio of titers obtained from treated and untreated samples; a high-avidity index allows the exclusion of a recent infection with an accuracy of time which is technique dependent. This test was initially developed through "in-house" methods (200) but has been commercially available in Europe for about 10 years and has been evaluated in numerous studies (138, 196, 304). In the United States, the first FDA-approved test (Vidas Toxo IgG avidity assay; bioMérieux) was approved in 2011. A high avidity ratio can exclude an infection acquired in the preceding 4 months with most commercial tests, ruling out an infection acquired during gestation when the test is performed during the first trimester of pregnancy. In routine practice, avidity testing allows the avoidance of unnecessary spiramycin treatment in pregnant women and unjustified long-term repeated follow-ups of fetuses and infants, provided that the test is performed before the fourth month of gestation. A U.S. study evaluated avidity testing in early pregnancy and showed that infection during gestation could be ruled out for 74 of 125 women (56%) with IgM detection and that correct interpretation by an expert from a reference laboratory reduced the rate of unnecessary abortions by 50% (205). However, when the avidity index is low or intermediate, the interpretation is ambivalent, since it cannot exclude an infection acquired in the preceding 4 months but also cannot prove that it is recent, unless the index is extremely low. From this standpoint, it should be kept in mind that treatment delays IgG avidity maturation (202). In a study by Petersen et al. (253), the mean IgG avidity index determined during the 16 weeks following infection was lower in treated pregnant women than in untreated nonpregnant patients (0.092 versus 0.149; $P < 0.01$). In that same study, only 2 out of 103 sera from treated women reached a high avidity index after 4 months, underlining again the potential impact of treatment and in accordance with other studies using other assay devices (138, 225). Another way to appreciate the age of infection resides in the kinetics of IgG titers analyzed in two serum samples obtained at 3-week intervals in the absence of any specific therapy. Rising IgG titers are suggestive of an infection acquired less than 2 months before the earlier sample, but one must be aware that treatment may also reduce or abolish the increase of IgG titers (202). Combinations of different diagnostic tools and clear-cut interpretations are necessary to achieve an accurate dating of infection and safe counseling for pregnancy management (Fig. 5). Proper serologic interpretation is critical to avoid the unnecessary worry of women with persisting IgM from a past infection, and laboratories that are not skilled in this field should refer samples to reference laboratories, as the consequences of misdiagnosis can be heavy.

A continuous effort has been made in the field of research and development of *in vitro* diagnoses, and opportunities for other methods for dating infections could emerge in the future. Giraldo et al. developed a capture ELISA to detect anti-glycosyl-inositol-phospholipid IgM that was mostly associated with recent infection but showed cross-reactivity with anti-*Plasmodium* IgM in 2 of 30 patients with malaria (150). Moreover, many recombinant proteins from *T. gondii* have been synthesized and tested for sensitivity and specificity in IgG ELISAs (194). Some of them displayed an interesting avidity response pattern, allowing a better performance than crude antigens for discriminating between chronic and acute infections, but their interest to clinical practice remains to be confirmed with larger series (28, 257).

Serologic Screening and Consequences for Clinical Management of Pregnancy

Some European countries have a policy for the prevention of congenital toxoplasmosis and undertake a systematic serologic screening of pregnant women, which is partially or punctually followed by other countries. In France, a first serology is performed before the end of the first trimester of pregnancy, and in the case of IgM detection, efforts are made to obtain reliable conclusions about the serologic status, with the testing of samples being repeated until a final interpretation is made. In the case of a negative result, serologic testing is repeated every month until delivery. A final examination is highly recommended 2 to 3 weeks after delivery to verify the absence of peripartum infection, which would have gone unrecognized, since antibody production would not yet have begun (201).

When recent infection is highly suspected at the beginning of pregnancy or when serologic conversion is observed, the patient must be referred to a reference center for confirmation, an accurate dating of infection, the validation of the indication of amniocentesis, and clinical counseling.

Diagnosis of Congenital Toxoplasmosis

Prenatal diagnosis and follow-up of pregnancy. When maternal infection acquired during pregnancy is clearly established or highly suspected, the current practice is to treat the mother with spiramycin until delivery and to propose a prenatal diagnosis (Fig. 6). Moreover, ultrasound surveillance is scheduled every month to monitor fetal development carefully. An amniotic fluid puncture is performed after 16 weeks of gestation and at least 4 weeks after maternal infection. Prenatal diagnosis relies mostly on the PCR-based detection of parasite DNA, but in most reference centers, amniotic fluid is also inoculated into mice. This *in vivo* assay relies on the detection of an antibody response in the animals by the examination of serum samples drawn 4 to 6 weeks after inoculation. This was the first means of parasite detection in the late 1980s (85), but because of the long response time and the lower sensitivity than that of PCR, it is now reserved mostly for strain isolation for epidemiological purposes. However, it can be punctually useful in cases of a nonconclusive PCR result, for the rare occurrence when inhibitors of *Taq* polymerase are detected.

Several PCR gene targets have been used over the years, with various performances in relation to the use of repeated or single target genes and the means of revealing amplification products (46, 53, 65, 295). The heterogeneity of PCR assays was clearly shown in a European study (250). The history of PCR use to detect *T. gondii* was extensively reviewed by Bastien (26). Most longitudinal studies reporting the sensitivity of prenatal diagnosis by PCR

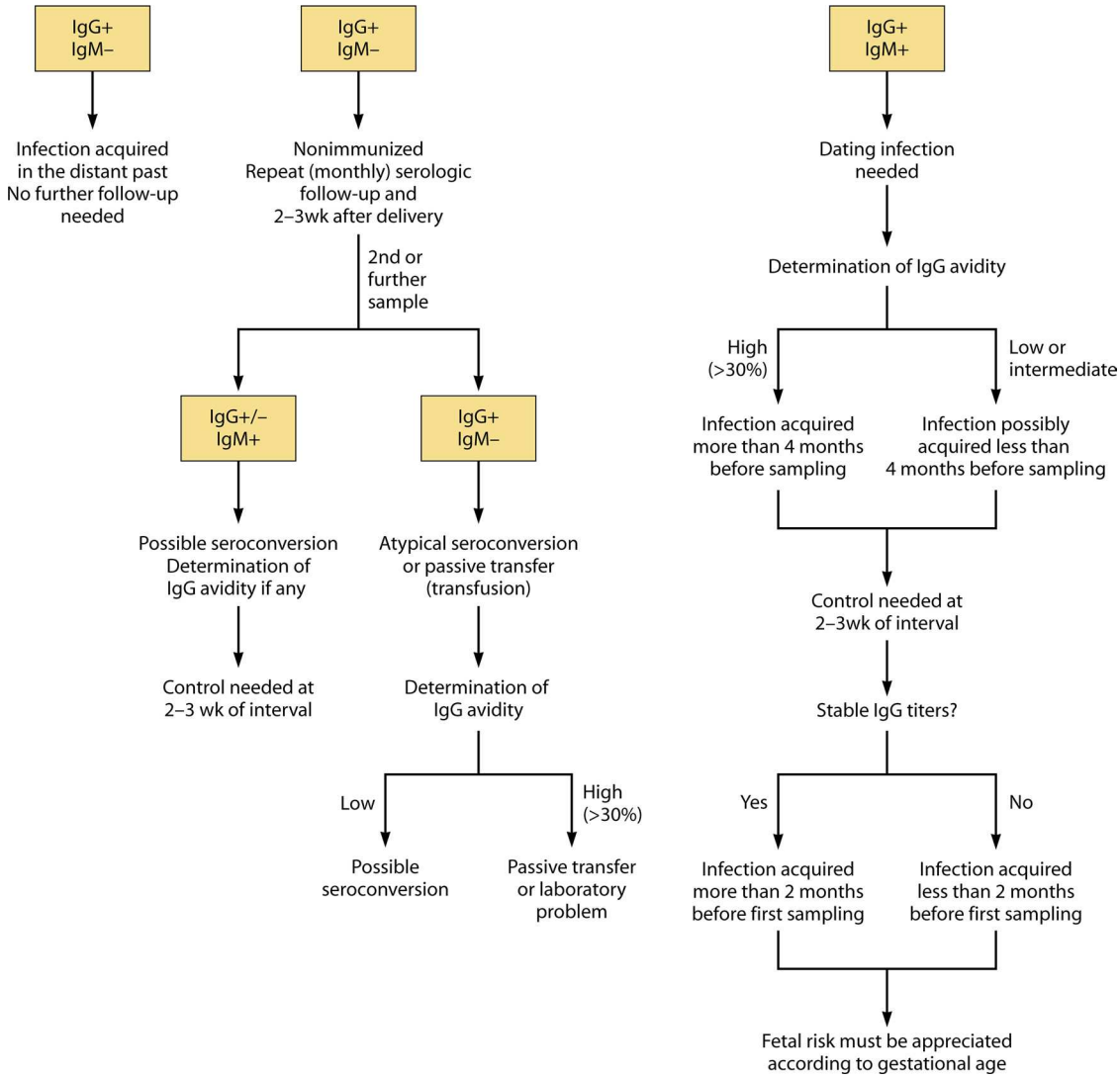

FIG 5 Interpretation of *T. gondii* serology in pregnant women.

for routine diagnosis were conducted in the late 1990s or early 2000s, with most of them targeting the 35-fold-repeated B1 gene. The sensitivity rates varied mainly from 65% to 80% using conventional PCR techniques (127, 277, 284), but multicenter studies pointed toward great differences between centers (317). To date, real-time PCR technology has replaced conventional PCR in routine practice and has probably contributed to a gain in sensitivity, but retrospective data comparing the use of the same target gene in a conventional PCR assay and in a quantitative PCR assay in large series are lacking. In addition, such analyses are complicated by the fact that extraction techniques and amplification reagents have simultaneously evolved over time and contribute largely to the efficacy of the PCR assay (340). Furthermore, in 2000, Homan et al. (173) described a new sequence target, REP-529, which is repeated 200- to 300-fold in the *Toxoplasma* genome and promised better performance. In France, where prenatal diagnosis has been routine for 2 decades, a particular effort has been made to inventory and evaluate the performances of PCR techniques used by reference centers (27, 310). These multicenter studies have objectified the ongoing widespread use of the REP-529 real-time PCR and pointed toward its higher sensitivity than that of B1 (310; F. Robert-Gangneux et al., presented at the 17th European Congress on Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 19 to 22 April 2008). A recent prospective multicenter clinical study evaluated the performance of the REP-529 real-time PCR and showed that parasites could be detected in amniotic fluid from 47/51 (92%) infected fetuses, thus confirming the high sensitivity of prenatal diagnosis using this target (332). These data are in agreement with national data collected annually in France since 2007 by the Centre National de Référence de la Toxoplasmose through the national surveillance network of experts from reference laboratories accredited for prenatal diagnosis (http://www.chu-reims.fr/professionnels/cnr-toxoplasmose-1/), which yielded a sensitivity of prenatal diagnosis of about 90%. It is now acknowledged that the false-negative results are probably the result of very low parasite densities in amniotic fluid (283, 310) or probable delayed parasite transfer through the placenta, rather than technical limitations. Indeed, the sensitivity of prenatal diag-

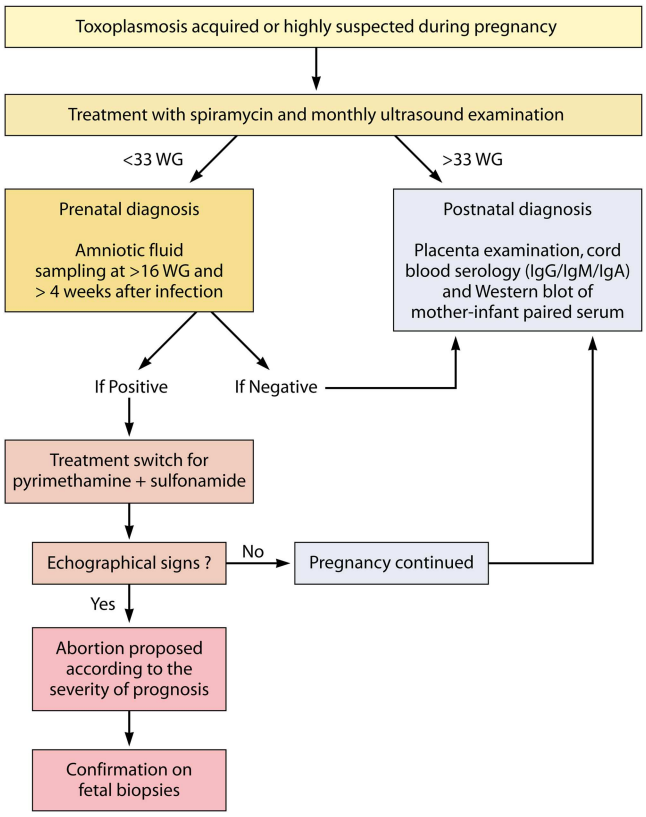

FIG 6 Management of pregnancy with confirmed or highly suspected pergestational toxoplasmosis. WG, weeks of gestation.

nosis is lower for maternal infections acquired during the first trimester than for infections acquired later, suggesting that vertical transmission could occur after amniotic fluid sampling (284, 332). In addition, early maternal treatment could contribute to reducing the parasite burden detected in the fetal compartment, which could explain the low numbers of parasites quantified in amniotic fluids. Quantitative PCR can be used as a prognostic marker, since higher *T. gondii* concentrations in amniotic fluid were shown to be correlated with clinical signs in fetuses or neonates (283). However, results from multicentric studies showed a lack of reproducibility of parasite quantification, particularly with low parasite burdens, and called for a standardization of the techniques (310).

What emerges from several studies is that PCR has a negative predictive value of nearly 100% for maternal infections in the first or second trimester of pregnancy (284, 332). On the other hand, a positive PCR, if performed in an accredited reference laboratory using general guidelines to avoid contamination, has a positive predictive value (PPV) of 100%.

In countries where screening policies include prenatal diagnosis, a switch of treatment from spiramycin to a pyrimethamine-sulfonamide (sulfadoxine or sulfadiazine) is performed in the case of a positive antenatal result (Fig. 6). There may be variations in this protocol; e.g., when infection occurs during the third trimester of pregnancy, therapy with pyrimethamine-sulfonamide may be prescribed immediately, and prenatal diagnosis may not be performed, according to the stage of gestation and depending on local practice. Medical abortion is usually reserved for cases with severe fetal abnormalities detected during ultrasound surveillance. The efficacy of prenatal treatment has been largely debated by contrary studies and is described in more detail in the last section of this review.

Postnatal diagnosis of congenital toxoplasmosis. The postnatal screening of neonates is an approach complementary to prenatal diagnosis or can be an alternative measure in countries where the serologic screening of pregnant women is not implemented.

Even with prenatal serologic screening, an analysis of amniotic fluid may not be performed for a large proportion of infected fetuses (22 to 52%), corresponding mostly to serologic conversion that occurred at a late stage of pregnancy (34, 275, 277, 332). Therefore, neonatal screening is critical to diagnose infection in this setting as well as to compensate for the few false-negative results of antenatal diagnoses.

(i) Follow-up protocol for newborns. At birth, the neonate undergoes a complete clinical and neurological checkup. A transfontanellar ultrasound examination is also performed to detect cerebral calcifications and may be supplemented by a computed tomography (CT) scan in the case of abnormalities, although its superiority has not been clearly demonstrated. An examination of the ocular fundus takes place during the first week of life and is repeated every 3 or 4 months according to local practice. Follow-up of neonates is essential when prenatal diagnosis was not performed or was negative, and efforts are made to detect biologic signs of fetal infection. There are two complementary strategies to diagnose congenital infection at delivery, i.e., (i) parasite detection in placenta or cord blood serum and (ii) serologic analysis of neonate serum with the aim of demonstrating the presence of specific antibodies which would be evidence of an *in utero* infection (Table 4). For this purpose, cord blood serum is recovered, and serologic testing of the neonate is repeated at 1 month of life and then every 2 or 3 months to monitor the decrease in levels of maternally transmitted antibodies, which usually disappear within 5 to 8 months. The different diagnostic tools and expected results in cases of infection are summarized in Table 4.

(ii) Serologic testing. Serologic diagnosis commonly relies on the detection of specific antibody isotypes that are produced by the neonate. The detection of specific IgM or IgA antibodies, which cannot cross the placental barrier, is a key marker of fetal infection but requires further confirmation when detected in cord blood serum, as it can reflect contamination with maternal serum at delivery. All serologic assays are not validated for IgM detection in cord blood or newborn serum, and few are available for IgA detection. The most sensitive one in this setting is an immunocapture test, the ISAGA. Nonetheless, the sensitivities for IgM and IgA detection do not exceed 70% and 65%, respectively, ranging from 41 to 70% for IgM in most studies (34, 149, 235, 277, 332). The poor detection of specific IgM and IgA in neonates appears to be influenced by maternal treatment, as shown by a multicenter European study (235), and more specifically, in a French series where all women who seroconverted were treated as usual practice, it was reported that IgM sensitivity was significantly lower when mothers were treated with pyrimethamine and sulfonamide than when they were treated with spiramycin (275). The combination of IgA detection and IgM detection was shown to increase the rate of diagnosis of infected neonates (33, 277, 332), but overall, what determines the detection of these isotypes at birth is the time of maternal infection, since IgM and IgA are more likely to be detected in newborns whose mothers seroconverted during the third

TABLE 4 Biological techniques used and their interpretation for diagnosis of neonatal congenital toxoplasmosis

Technique used	Sample	Frequency of sampling	Interpretation	
			Positive if:	Negative if:
Mouse inoculation[a]	Placenta[b] Cord blood	At birth	At least one positive mouse with brain cysts detection	Negative serology in all mice
PCR	Placenta[b] Cord blood	At birth	Positive PCR	Negative PCR
Serology (IgG, IgM, IgA)[a,c]	Cord blood serum	At birth	IgM detection[d] IgA detection[d]	No IgM detection No IgA detection
Western blot with paired mother-cord blood samples[a]	Cord blood serum and mother serum (at delivery)	At birth	Specific IgG or IgM pattern in the newborn	Identical IgG patterns (transmitted antibodies), no IgM in the newborn
Serology (IgG, IgM, IgA)[c]	Infant serum	At 1 mo of life and then every 2 or 3 mo	IgM detection IgA detection Absence or decrease of IgG titers over 6 mo or persistence over 12 mo of age	No IgM detection No IgA detection Clearance of maternal IgG under age of 1 yr
Western blot on paired mother-child samples[a]	Infant serum and mother serum (at delivery)	At 1 mo and 2-3 mo of life	Specific IgG or IgM pattern in the newborn	Identical IgG patterns (transmitted antibodies), no IgM in the newborn

[a] Should be done by reference laboratories.
[b] In rare instances, the detection of parasites in placenta may occur in the absence of congenital infection.
[c] IgM and IgA detection by immunocapture or, better, by an immunosorbent agglutination assay (ISAGA).
[d] Attention must be paid to the possible contamination of cord blood by maternal serum at delivery. A positive result needs to be checked at 1 week of life.

trimester of pregnancy (33), an observation which is consistent with a transient synthesis of fetal IgM or IgA and its disappearance before delivery when maternal infection occurred at an early stage.

As maternal IgG is passively transferred *in utero*, the simple quantitative assay is of no help in the diagnosis of fetal infection, as the IgG titer reflects mostly the amount of transferred antibody and can easily hide a low level of synthesis of IgG by the neonate himself. Therefore, a qualitative analysis is needed to differentiate between maternal antibodies and antibodies synthesized by the infected neonate, which are likely to recognize different *T. gondii* antigens. A comparative analysis of mother- and neonate-specific IgG may help provide serologic evidence of fetal infection, particularly when no IgA or IgM is detected. Western blotting (WB) (268) and an enzyme-linked immunofiltration assay (ELIFA) (258) both allow a qualitative analysis of specific IgG or IgM by a comparison of band patterns or precipitin patterns, respectively, from paired mother-newborn sera with an equal sensitivity (259). Several studies analyzed the diagnostic performance of WB and reported sensitivities of 48 to 50% and 78 to 82% for IgG detection at birth and within the first 3 months of life, respectively (274, 320). The combination of WB detection of IgM and IgG allowed a gain in sensitivity, reaching 65 to 79% and 83 to 94% at birth and at 3 months of life, respectively (271, 274, 320). The combination of ISAGA and WB yielded even better results in those studies. However, much attention must be paid to the reading and interpretation of the band patterns. Indeed, interpretations of similar mother-infant patterns showing some bands of greater intensity for the infant serum must be done with caution, since they can be observed for noninfected newborns. Such variations in estimations between readers could account for specificities below 100% in some series (271, 320). Aside from this restriction, WB was the earlier test allowing the diagnosis of congenital infection in 3/23 and 1/17 asymptomatic newborns in studies by Tissot-Dupont et al. (320) and Robert-Gangneux et al. (274), respectively. In these cases, where the detection of IgM, IgA, and parasites was negative at birth, it would have taken several months to observe the absence of a decrease of the IgG titer after repeated testing if WB had not been available. Therefore, this test has provided significant advances in the early diagnosis of congenital toxoplasmosis, particularly in the first 2 or 3 months after birth. Notably, it offers the advantage of easily confirming the contamination of cord serum with maternal serum by yielding identical IgM patterns, thus avoiding the urgent checking of the neonate and needless worry for the mother. However, considering the high cost of this test, now commercially available and thus warranting a reproducibility of manufacture, it should be reserved for cases where the detection of cord IgM needs to be confirmed or where an IgM ISAGA is negative.

(iii) Parasite detection. The parasitological examination of placental tissue is one of the usual biologic tools used to diagnose a congenital *Toxoplasma* infection at birth, along with the serologic screening of the newborn. As for prenatal diagnosis, the demonstration of parasites in the placenta relies on mouse inoculation or DNA detection by PCR. Most retrospective studies reported that the examination of the placenta has a 42 to 71% sensitivity and a 92 to 100% specificity (34, 134, 137, 235, 275, 277), depending on the techniques used. The combination of both methods yields a better sensitivity, reaching 74% in one study (137). Similarly to IgM serologic screening, a decreased sensitivity for parasite detection by mouse inoculation was reported in some studies when mothers were treated with a regimen combining pyrimethamine and a sulfonamide (16, 20, 22). Despite heterogeneous results for

sensitivity, the positive predictive value (PPV) was high, ranging from 91 to 100% for the mouse assay (134, 275); thus, a positive placenta result is a parameter of critical importance for the management of neonates with suspected congenital infection. A positive result obtained only by PCR is more debatable, since in some studies, parasite DNAs could be detected in a few cases where congenital infection was ruled out (33, 134, 275). Therefore, the PCR result cannot be fully reliable by itself and should be confirmed by another criterion in favor of infection. The sensitivity of the mouse assay is particularly high when maternal infection occurred during the third trimester of gestation, which could be related to a shorter exposure to maternal treatment. In these mothers at a late stage of gestation but with a high likelihood of vertical parasite transmission, an examination of the placenta compensates for the frequent lack of a prenatal diagnosis. In our hands, in 3 of 27 infected neonates born alive, the detection of parasites in the placenta was the only evidence of congenital toxoplasmosis at birth and during the first 3 months of life, since the prenatal diagnosis was negative or was not performed and IgM was not detected in the neonates (275). A similar observation was reported by Fricker-Hidalgo et al., who diagnosed 3 of 33 infected neonates by means of placental examination only (137). Such data emphasize the clinical relevance of a positive placental sample, which can contribute to an early diagnosis in situations where prenatal screening is not performed or is negative.

(iv) New hopes for the future. An IFN-γ release assay was described recently, which could open a new complementary approach for the postnatal diagnosis of congenitally infected infants (54). This test is based on the measurement of IFN-γ concentrations by an ELISA after the *in vitro* stimulation of whole blood cells with crude *Toxoplasma* antigens. The authors of that study evaluated this method with 62 infants under 1 year of age, 17 of whom were congenitally infected, and reported a sensitivity of 94% and a specificity of 98% (54). This test was the only criterion for congenital infection in 2 infants between 3 weeks and 3 months of age. This type of assay has already proven useful for the diagnosis of *Mycobacterium tuberculosis* infection (184) and could be part of future screenings for congenital toxoplasmosis if its reliability is confirmed. However, its implementation in routine use will require standardization, since there is no commercial test available for this indication.

Diagnosis of Toxoplasmosis in Immunocompromised Patients

Parasite detection. Since acute toxoplasmosis in immunocompromised patients can be rapidly lethal, its diagnosis is an emergency. Whether serology is essential to estimate whether the patient is at risk for a reactivation of infection, evidence of evolutive infection is provided by the demonstration of tachyzoites in fluids or tissues by PCR or microscopic examination (Table 3). Although a direct examination of Giemsa-stained tissue sections or smears is the fastest and cheapest means of diagnosis, it frequently lacks sensitivity. An attempt to isolate the parasite strain can be undertaken by mouse inoculation or cell culture. Various samples can be collected according to clinical signs and the type of immunosuppression. In transplant patients, where disseminated toxoplasmosis is frequent, parasites can be detected in bronchoalveolar lavage (BAL) fluid, blood, bone marrow aspirate, cerebrospinal fluid (CSF), or virtually any biopsy specimen from a deep site. For these patients, the control of *Toxoplasma* disease may require a reduction of immunosuppressive therapy, which can be monitored by the measurement of the decrease of parasite loads using quantitative PCR (248).

During cerebral toxoplasmosis, parasite DNA can be detected in CSF and blood with sensitivities of 33 to 65% (57, 241, 247, 297) and 16 to 23% (130, 199, 273), respectively. However, these data are drawn from studies evaluating *Toxoplasma* DNA detection, which have been conducted with HIV patients from Europe or Northern America before the use of HAART and before the availability of quantitative PCR methods. Therefore, these results must be interpreted with some caution for several reasons. First, it is likely that nowadays, molecular diagnosis has gained in sensitivity, similarly to prenatal diagnosis, which benefited from new-generation PCR methods. However, at the same time, the use of HAART has diminished considerably the incidence of toxoplasmic encephalitis in these countries, thus limiting the collection of new data. Second, current knowledge of strain genotypes and their virulence should lead us to reconsider the sensitivity of PCR for blood according to strain virulence, which conditions its capacity for multiplication and dissemination. Recent studies conducted with HIV-infected patients in Brazil and Cuba pointed toward PCR sensitivities of 69 to 83% (14, 240) and 80% (58) in CSF and blood, respectively. These high sensitivities could indeed be in agreement with the higher virulence of parasite genotypes circulating in South America, but technical pitfalls could also explain such high rates. Indeed, a more recent study did not confirm these data, since those authors found only 29/82 (35%) and 1/85 positive CSF and blood samples, respectively (63).

Place of serologic testing. Serologic testing is useful (i) as an exclusion criterion, except for HSCT patients, when it is negative for a patient with symptoms consistent with acute toxoplasmosis or (ii) as a monitoring indicator, mainly for solid-organ transplant patients, prompting further investigations in cases of strong increases in IgG titers. In rare instances, it can also provide evidence for recent infection acquired orally (136) or, more rarely, from a transplanted organ. For transplant patients, serologic test results need to be interpreted with the knowledge of their antibody status prior to transplantation. Indeed, IgM may reappear during the course of a reactivation of infection (136), and on the contrary, the antibody response may be abolished, particularly in HSCT patients (79). Regular serologic testing is common following heart transplantation, but attention must be paid to the interpretation of rising IgG titers, since serologic reactivation is frequently observed, independently of any clinical reactivation (211). For HIV-infected patients, Derouin et al. reported that high IgG titers could be predictive of the occurrence of toxoplasmic encephalitis when $CD4^+$ cell counts fall under 200 cells/μl (80), which is in accordance with a subclinical reactivation of dormant cysts before clinical manifestations. However, the delay between the rise in antibody titers and the occurrence of TE is not well defined, since it depends closely on the evolution of the immune balance of each patient.

Diagnosis of Retinochoroiditis

The diagnosis of retinochoroiditis relies primarily on an ophthalmological examination. The presence of typical lesions (white focal lesions often associated with a vitreous inflammatory reaction), together with *Toxoplasma* seropositivity, calls for specific anti-*Toxoplasma* treatment, further confirmed by a good clinical response. However, biological examinations are necessary for

some patients with atypical ocular lesions or those in whom the response to anti-*Toxoplasma* therapy is inadequate. Laboratory methods include parasite detection and analysis of local antibody production in ocular fluids, with both approaches being complementary. PCR detection of parasite DNA in aqueous humor (AH) or vitreous fluid has been reported with variable successes even using quantitative PCR techniques, as its sensitivity was evaluated to be 16 to 55% (122, 143, 231, 314, 327). It has been suggested that *Toxoplasma* DNA is more likely to be detected in aqueous humor from immunocompromised patients, but prospective data are scarce and the published series are small, thus not allowing the drawing of clear conclusions. Westeneng et al. (338) reported positive PCRs for 4 of 10 patients with uveitis with various causes of immunodeficiency, whereas Talabani et al. (314) found parasite DNA in 8/11 patients (73%), for whom a positive PCR result was the only positive test in 4 cases. In a series of atypical retinochoroiditis by Fardeau et al., 8/9 patients with a positive PCR result were immunocompromised, but at the same time, PCR failed to detect 9 patients who responded to anti-*Toxoplasma* treatment (120).

The detection of a specific antibody response in ocular fluids is a useful complementary tool for the diagnosis of ocular toxoplasmosis. Ocular fluid antibody production can be quantitatively or qualitatively determined by comparison to serum IgG titers. Abnormal amounts of anti-*Toxoplasma* antibodies in aqueous humor can be evidenced by the calculation of the Goldmann-Witmer coefficient (GWC), with a sensitivity of up to 81% (122) but more frequently around 50% (143, 279, 314). The GWC is defined as follows: [anti-*Toxoplasma* IgG (IU/ml) in AH/anti-*Toxoplasma* IgG (IU/ml) in serum] × [serum total IgG (g/liter)/AH total IgG (g/liter)]. An alternative method using mumps virus antibody titers as a comparator to evaluate the anti-*Toxoplasma* antibody load in AH was proposed by Turunen et al. (321). It has been underlined that repeated sampling at intervals of several weeks allowed a gain in the sensitivity of the GWC, which rose from 57% to 70% in one study (143). However, the optimal time for eye sampling is debatable, since other authors attributed a low PCR performance to late sampling (327). The length of the interval between the onset of clinical symptoms and paracentesis also seems to be critical for the detection of specific band patterns by Western blot analysis of serum and paired AH samples, with better results being obtained for intervals from onset to sampling of more than 30 days (314). The sensitivity of WB is similar to that of the GWC, as it ranges from 53 to 81% (122, 279, 314, 327), but its specificity was higher (>95%) in most studies, since it is less influenced by inflammation and the rupture of the blood-retina barrier. The detection of specific IgA in AH has also proven to be useful, either as a quantitative marker of local antibody synthesis by computing a ratio between serum and AH titers (195) or as a qualitative marker by comparative WB patterns (142), with sensitivities of 63% and 76%, respectively.

Data obtained from several large series of patients clearly demonstrate that combining techniques improves the sensitivity of biological diagnosis, which can reach 83 to 85% when the GWC, PCR, and WB are combined (314, 327). Some authors proposed an algorithm to decide which techniques to apply when a small volume of AH is available, according to the patient's own characteristics and the time of AH puncture (314): (i) priority for the use of real-time PCR during the 10 days following the onset of symptoms, especially if the patient is immunocompromised or if the total size of the foci is large (>2 optic disc diameters); (ii) the use of the GWC beyond 10 days if old scars are present and/or if the reaction in the anterior chamber is mild to severe, associated with PCR if the total size of foci is large (>2 optic disc diameters); and (iii) WB, which would be optimal when sampling occurs more than 30 days after the onset of symptoms. The observations reported by Labalette et al. for 27 patients older than 50 years of age suggest that the combination of AH antibody load determinations and PCR improves the sensitivity of diagnosis independently of the time from onset to diagnosis and the size of the lesions. However, PCR alone is more sensitive for patients with lesions equal to or larger than 3 disk areas (195).

As AH puncture remains an invasive procedure for investigation, the detection of parasite DNA in whole blood would be an attractive alternative. However, attempts to demonstrate circulating DNA during episodes of retinochoroiditis have been successful in a limited amount of cases, mostly in immunocompromised patients (56). The source of circulating parasites is uncertain, as it could originate from the eye as well as from other peripheral sites. In immunocompetent patients, current hypotheses on the pathophysiology of toxoplasmic retinochoroiditis are in favor of a reactivation of encysted parasites in the retina or the choroid, with limited dissemination. However, this concept could be questioned in the near future in the light of parasite strain genotypes and virulence. Indeed, a case of congenital transmission following a reactivation of toxoplasmic retinochoroiditis in an immunocompetent pregnant woman in Brazil was recently described (15). This observation shows that parasite redissemination can happen during reactivation events and is consistent with the particular virulence associated with genotypes from South America. It could also explain the unexpected results reported in a recent Brazilian study, where 7/8 non-HIV-infected patients had a positive PCR on peripheral blood (305). Extensive studies of larger series are therefore welcome to validate the prevalence of circulating parasites in immunocompetent patients living in South America and possibly to reconsider the sensitivity of diagnostic tools for these patients.

HOW TO AVOID OR REDUCE THE BURDEN OF TOXOPLASMOSIS: PREVENTION AND CONTROL MEASURES

Primary Prevention, or How To Avoid Infection

Hygienic measures are paramount to avoiding infection. Health education measures to prevent primary *T. gondii* infection are listed in Table 5 and are drawn directly from the acquired knowledge of the intrinsic resistance and biological characteristics of the infective stages of *T. gondii*. Persons should be advised that they should wash their hands after contact with raw meat, after gardening or other external activity with contact with soil, and after having close contact with a cat. In addition, persons should wash fruits and vegetables (especially those growing in contact with soil) thoroughly before eating them raw. If the person owns a cat, the litter box should be changed every 2 days, preferably by another person, or alternatively, persons should wear a mask and gloves when changing the litter box. Persons should be encouraged to keep their cats inside and feed them only canned or dried commercial food.

The spread of such measures depends on the health care policy applied in a given country. Physicians are at the first line to comprehensively explain the preventive measures to women who are

TABLE 5 Basis for hygienic measures for prevention of toxoplasmosis

Source of infection	Type of risk	Prevention measure(s)
Oocysts and cat feces	Direct contact with cat feces; oocysts become infectious only 2–3 days after shedding, shedding usually occurs only once during a cat's life, duration of shedding is about 2 wk, oocysts are killed within 1–2 min by heating to 55°C–60°C, and oocysts are resistant to chemical disinfectants such as sodium hypochlorite	Wash hands carefully after stroking a cat, wear gloves when changing cat litter, change the litter frequently and wash tray with hot water (>60°C), avoid putting cat litter in the kitchen, and feed cats dried or canned food
Oocysts in the environment	Soil contact for gardening, playing, or other outdoor activities; oocysts can survive more than 1 year in a moist environment at 4°C, 106 days at −10°C, 32 days at 35°C, and 9 days at 40°C	Wash hands thoroughly and brush nails after any outdoor activities in contact with soil, and wear gloves for gardening
	Consumption of unfiltered water (raw surface water, reservoir, wells, recreation areas)	Prefer mineral water to tap water (in countries where the water network is supplied mainly by surface water)
	Oocysts can survive for long periods of time in water and resist freezing and moderately high water temperatures, chlorination and ozone treatment, in seawater, and in various species of shellfish	Avoid raw oysters, clams, and mussels, and avoid occasional ingestion of water (lakes, rivers) during recreation activities
	Raw vegetables or fruit consumption	Thoroughly wash vegetables, fruits, and herbs eaten raw, especially if they grow close to the ground, and avoid raw vegetables at restaurants
Tissue cysts in meat	Meat consumption or manipulation; any type of meat may be infected, with sheep, goats, and pigs from organic outdoor production systems and wild game being at the most risk; cysts are killed immediately at 67°C and after at least 3 days at <−12°C, depending on the thickness of the piece of meat; cysts can survive in refrigerated meat for up to 3 wk, for >11 days at −6.7°C, and for about 4 min at 60°C and 10 min at 50°C	Cook the meat well done (oven, pan) or stew; avoid microwave cooking; eat meat frozen for at least −20°C during at least 15 days; and wash hands, knives, any containers, and table thoroughly after meat manipulation or cutting

pregnant or plan to be pregnant. Oral counseling should be accompanied by written information for optimal retaining of the information throughout pregnancy. There is evidence to suggest that health education approaches may help reduce the risk of congenital toxoplasmosis, but evaluation studies of educational policies worldwide are lacking (151). It could be deduced from an epidemiological case-control study conducted in France that cat-related prevention measures are probably well known, since cat owners were not more likely to acquire toxoplasmosis than others (23). Indeed, cat owners are usually warned of the risk associated with cleaning the litter box or having close contact with cats or kittens and take appropriate measures. Conversely, in that study, the risk for primary acquired infection was highly associated with the consumption of undercooked meat or with rural living, suggesting acquisition through contaminated soil (gardening or working in a rural environment) (23). Undercooked meat was the main risk factor for infection of pregnant women in another European case-control study, leading those authors to propose the clear labeling of meat at risk according to farming and processing methods (61). As freezing meat during at least 3 days at −12°C usually allows the killing of cysts from pork meat (91), the purchase of frozen meat (at least −20°C) may be recommended to people who cannot think of eating well-done pieces of beef or lamb, on the basis that long periods of freezing at low temperatures should kill all cysts.

The risk associated with water consumption is less known and was recently reviewed by Jones and Dubey (180). More attention is indeed being given to the consumption of water, particularly untreated or unfiltered water, in countries where surface water is the main source of drinking water and where water-filtering systems are malfunctioning or use water filters with a diameter that is too large. Several outbreaks of waterborne toxoplasmosis have been reported both in developing countries (Brazil and India) (167, 245) and in developed countries (North America and Poland) (40), suggesting that prevention measures should now stress this specific risk and recommend the consumption of mineral water for pregnant women. Moreover, it has not been excluded that the ingestion of contaminated water from lakes or rivers during recreational activities could be a source of *Toxoplasma* infection, which may explain the large proportions of cases of unexplained toxoplasmosis in pregnant women, as shown in a study in the Northern United States (41). From a general point of view, given the potentially huge contamination of the environment by oocyst spreading (180), one must keep in mind all hygienic measures in relation with external activities and should wash hands thoroughly after gardening or other recreation activities.

An introduction of new prevention messages concerning the risk due to more recently recognized sources of contamination (unpasteurized goat's milk and raw shellfish, etc.) was also proposed (179).

The same guidelines of primary prevention can be given to immunocompromised patients who are seronegative, to avoid *Toxoplasma* contamination. Recent knowledge of strain virulence should be taken into consideration, and these recommendations

should now also be provided to travelers who are visiting countries where atypical strains predominate, even if they were previously immunized.

Various Approaches for Screening, Prevention, and Control of Congenital Toxoplasmosis

Screening and treatment of pregnant women to reduce parasite transmission. Serological screening of pregnant women is not the rule and differs among countries according to the prevalence of *Toxoplasma* and health care policies. The high prevalence (>70%) observed in France in the 1970s motivated the implementation in 1985 of mandatory prenatal serologic screening during the first 3 months of pregnancy, which was strengthened in 1992 by the implementation of monthly repeated testing of seronegative women during pregnancy. In Austria and some other European countries (Belgium, Norway, and Italy, at least in some regions), a retesting schedule at 3-month intervals has been implemented, whereas other countries (Poland, Denmark, Sweden, and the United States) have no prenatal screening program. In Switzerland, where the seroprevalence is intermediate (about 25%), the Swiss Working Group on Congenital Toxoplasmosis recently recommended the abandonment of the surveillance and prevention program, arguing for the low incidence and morbidity of congenital toxoplasmosis in this country, although this recommendation is not unanimously shared by all teams (311). In the absence of screening, hygienic measures are the keystone of prevention and should be largely disseminated to pregnant women. In countries with prenatal screening policies, serology should be prescribed as soon as the pregnancy is diagnosed so that the serologic status can be interpreted unambiguously and prevention measures can be given to seronegative patients early to avoid infection. Repeated serologic screening offers the option to start a specific treatment as soon as seroconversion is observed and to propose a prenatal diagnosis. However, independently of any critical consideration about treatment efficacy, there are two limitations to prenatal screening. First, a consequence of screening is the anxiety generated by the diagnosis of *Toxoplasma* infection in the mother, as highlighted by some authors (118). This anxiety may lead the couple to prematurely terminate the pregnancy but, on the other hand, can be greatly lightened in cases of negative amniocentesis results. Second, other authors reported difficulty in applying systematic screening and the possible lack of compliance of patients, limiting the efficacy of health policies (62). In addition, the risk of adverse events associated with amniocentesis must be weighed against the risk of fetal transmission associated with the time of pregnancy when maternal infection occurred. A meta-analysis reported prenatal diagnosis-associated risks of fetal loss ranging from 0.33% to 0.74% until 2002 and from 0.3 to 0.4% in recent cohort studies (189), which are lower than the rate of transmission of parasites during the first trimester of pregnancy but similar to or higher than the rate of transmission of parasites following periconceptional toxoplasmosis. Therefore, the disease burden of congenital toxoplasmosis may be diversely appreciated according to the health care system: the burden of morbidity is expected to be higher in countries without prenatal screening, but the burden of mortality is higher in countries with active prenatal screening (voluntary termination, medical abortion, or fetal loss due to amniocentesis) (163). Hence, decisions about the implementation of prenatal screening in a given country should be carefully considered and based on prevalence data, disease burden, technical resources, and diagnostic costs.

As part of primary prevention, the aim of diagnosing maternal infection through serologic screening is to treat the mother to avoid vertical transmission. Spiramycin has been used in France since the 1960s for the primary prevention of congenital toxoplasmosis (141), but a combination of pyrimethamine-sulfonamide in association with folinic acid can also be used after the first trimester of pregnancy. The rationale for the use of spiramycin relies on reducing the parasite burden in the mother (mainly bloodstream and placenta), since this molecule hardly crosses the placental barrier (157), whereas pyrimethamine-sulfonamide does so efficiently, as demonstrated by comparative dosages of these drugs in maternal and cord blood sera at delivery (55). However, over the last decade, contradictory results on treatment efficacy drawn from several epidemiological studies or meta-analyses of retrospective cohorts have opened a large debate questioning the pertinence of screening. A systematic review by Wallon et al. (334) included for analysis 9 out of 2,591 papers on congenital toxoplasmosis and concluded that there was an absence of a clear effect of treatment on reducing vertical transmission. However, some important parameters were lacking in some of those papers, i.e., a prolonged follow-up of newborns for >6 months (one study), the precise delay between maternal infection and the onset of treatment (all 9 studies), and the lack of adequate untreated controls, thus limiting the reliability of general conclusions on treatment efficacy. The delay to treatment is indeed a key point for the evaluation of treatment efficacy, as is an adjustment for the age of gestation at the time of maternal infection. The EMSCOT (European Multicenter Study on Congenital Toxoplasmosis) (145), a large meta-analysis of 1,208 mother-child pairs from 11 centers, found no effect of treatment on reducing parasite transmission, but those authors did not exclude that their analysis could lack power. Indeed, there are several biases in the interpretations of results from this large-cohort study, such as differences in treatment regimens, the low number of untreated controls (only 106 out of 1,208 patients), and, again, the possible long delay for treatment in cohorts from countries with serologic screening at 3-month intervals. In addition, in that study, the median treatment delay for women first treated with spiramycin was about 4 weeks, whereas it was >8 weeks for those first treated with pyrimethamine-sulfonamide, which could contribute to the decrease in the overall treatment efficacy, since the most powerful drug combination was given with a greater delay. Two other retrospective cohort studies failed to detect an effect of treatment on reducing parasite transmission, whatever the delay to treatment (128, 147). Nevertheless, a more recent large meta-analysis from the SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group (318) including 26 cohorts and 1,745 infected mothers (307 untreated) found weak evidence that treatment that started within 3 weeks of seroconversion reduced mother-to-child transmission compared with treatment started after >8 weeks. All authors agreed to conclude that definitive conclusions on prenatal treatment efficacy should rely on a large randomized controlled trial. However, due to ethical considerations, a trial including an untreated group cannot be undertaken in countries where screening and prevention are routine. To answer, at least partly, the debate on maternal treatment, a 3-year French national clinical trial (Toxogest [http://clinicaltrials.gov/ct2/show/NCT01189448]) was started in 2010 to compare the treatment efficacies of spiramycin and pyri-

methamine-sulfadiazine and should include 330 patients. The analysis of clinical outcomes in newborns and of transmission rates in both groups should help to answer the question of the role of both the delay of treatment and the therapy used according to the stage of pregnancy.

Prenatal screening and treatment to limit fetal damage. Apart from decreasing vertical transmission, the associated goal of prenatal treatment is to reduce fetal damage or newborn sequelae, provided that transmission has occurred. The efficacy of prenatal treatment to reduce clinical manifestations at birth can be evaluated with cohorts of patients benefiting from prenatal screening, but as discussed above, the evaluation often suffers from the lack of untreated controls and from the necessity to precisely date the maternal infection, since the severity of fetal infection, and, proportionally, the capacity for treatment to reduce sequelae, depends on the term of gestation at seroconversion. Again, different studies have yielded contrasting conclusions. The SYROCOT study found no evidence that prenatal treatment reduced the risk of clinical manifestations (318). However, two recent cohort studies showed that (i) any prenatal treatment (spiramycin or pyrimethamine-sulfonamide) reduced the risk of serious neurological sequelae (64) and (ii) any prenatal treatment reduced the risk of intracranial lesions, provided that treatment was given within the first 4 weeks following maternal infection (156). In addition, in the prospective EMSCOT (64), the number of infected fetuses needed to be treated to prevent 1 case was only 3 for maternal infections at 10 weeks of gestation. These findings hence support prenatal screening and are in agreement with the observation that the systematic screening and treatment of pregnant women have decreased the rate of severe congenital infections in France, where the rate of asymptomatic infected neonates is about 85% at birth (328). However, due to the lack of a national surveillance system at the onset of the prevention program, it is not possible to quantify precisely the gain in terms of disease burden since then. The impact of treatment on eye sequelae is less clear. Some studies did not find any beneficial effect of prenatal treatment on the number of eye lesions or episodes of recurrence after birth (131, 318), whereas others did (128, 190). Kieffer et al. reported on a series of 300 infected infants and described that the risk of developing a first retinochoroiditis before the age of 2 years was associated with a delay of >8 weeks between maternal infection and prenatal treatment (190). Wallon et al. reported the longitudinal follow-up of 327 infected infants (275 of whom were treated antenatally) who were treated with pyrimethamine and sulfadiazine during their first year of life (333). During a median follow-up time of 6 years, 24% of children developed at least one lesion of chorioretinitis, and 29% had one or more episodes of recurrence before the age of 10 years; 63% had normal visual acuity. However, from the data presented, it cannot be deduced how many of those with visual impairment were not treated antenatally.

Postnatal screening of neonates to promote early treatment. The postnatal screening of neonates has been implemented in some countries where prenatal screening was not considered to be a health priority, as is the case in Sweden, Denmark, Poland, and the United States (Massachusetts). The concept of newborn screening for early treatment to reduce long-term eye sequelae has been prompted by American cohort studies, which showed unexpected favorable outcomes for most infants who were treated continuously for 1 year with pyrimethamine-sulfadiazine, despite severe manifestations at birth, compared to untreated infants from previous series (159, 220, 221). Phan et al. (256) reported on 28 children with congenital toxoplasmosis who were diagnosed late and were not treated during their first year of life. Those authors described new chorioretinal lesions in 72% of these children during a mean follow-up time of 5.7 years; 52% of the children developed new eye lesions at an age of >10 years. These data are in contrast with data for European cohorts, where eye prognoses seemed better (45, 333). In the French series described by Kieffer et al. (190), only 12% of 300 infected infants treated for 1 year developed a first retinochoroidal lesion until the age of 2 years. However, French series present a confounding factor limiting analyses of the efficacy of postnatal treatment, since most babies also received prenatal treatment. Therefore, the answer regarding the effect of early postnatal treatment should come from countries where decision makers chose the option of neonatal screening only. But how does one obtain an adequate untreated control group? Such a definitive evaluation also needs a randomized controlled trial, with the same ethical limitations as those for prenatal treatment.

Freeman et al. observed a low incidence of retinochoroiditis among 281 infected infants (18%) identified by prenatal or postnatal screening and proposed a strategy of postnatal therapy based on clinical severity and prognosis (131). They suggested a short-course treatment (3 months) or no treatment for asymptomatic newborns who are at a low risk of developing retinochoroiditis but recommended treatment and careful monitoring of children with early eye manifestations and/or severe disease (intracranial lesions, serious neurological sequelae, and hepatosplenomegaly), who have a higher risk for new or worsening lesions. In Denmark, intervention studies since the onset of the national neonatal screening program estimated that the low burden of disease (1.6 per 10,000 live-born infants) and the lack of evidence of postnatal treatment efficacy did not justify the continuation of neonatal screening; hence, the Danish program was stopped in 2007 (287). The Danish protocol was based on a 3-month treatment of infected newborns, which failed to prevent the development of retinochoroidal lesions in children with or without previously detected lesions (296). However, it cannot be excluded that the duration of treatment may have been too short, particularly for symptomatic newborns at birth.

Screening for and Chemoprophylaxis of Toxoplasmosis in Immunocompromised Patients

Primary prevention aims to screen targeted patients to identify those who are at risk of acquiring a primary *Toxoplasma* infection, either naturally, i.e., oral infection, which can be prevented by hygienic measures, or through the transplantation of an organ from a seropositive donor, which can be prevented by chemoprophylaxis.

Secondary prevention relies on chemoprophylaxis and concerns immunocompromised patients who are seropositive for *Toxoplasma*, i.e., who have already acquired *Toxoplasma* infection in the distant past and are likely to have a reactivation of infection, according to the degree of immunodeficiency.

As for congenital toxoplasmosis, there is no consensus about serologic screening for immunodeficient patients. For HIV-infected patients, annual serologic testing is usually performed for *Toxoplasma*-seronegative patients to verify the immune status against the parasite, particularly in the case of an onset of clinical manifestations suggesting evolutive toxoplasmosis. Usually, practitioners refer to national

guidelines from scientific societies or national agencies. French and American recommendations can be found online at http://www.infectiologie.com/site/consensus_recos.php and http://aidsinfo.nih.gov/contentfiles/Adult_OI000999.pdf, respectively, but others are also available. The prophylaxis of TE in *Toxoplasma*-seropositive AIDS patients is rather consensual, and trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis should be administered when the $CD4^+$ T cell count falls below 100 cells/μl. A daily dose of the double-strength tablet (160 mg–800 mg) of TMP-SMX is recommended as the preferred regimen, since it is also suited for *Pneumocystis jirovecii* (formerly *carinii*) pneumonia (PCP) prophylaxis. TMP-SMX (160 mg–800 mg) three times weekly or TMP-SMX (80 mg–400 mg) daily are alternatives to improve tolerance. If patients cannot tolerate TMP-SMX, the recommended alternative is dapsone-pyrimethamine plus folinic acid, which is also effective against PCP. Treatment with pyrimethamine-sulfadoxine twice weekly also proved to be effective (298), but cutaneous adverse effects limit its use. Atovaquone with or without pyrimethamine-folinic acid can also be considered, provided that it is properly absorbed in the digestive tract (307). Prophylaxis should be discontinued for patients who have responded to HAART with an increase in $CD4^+$ cells counts to >200 cells/μl for at least 3 months. *Toxoplasma*-seronegative persons should be retested annually for IgG antibody to *Toxoplasma* to determine whether they have seroconverted and are therefore at risk for TE.

The decision to screen for *Toxoplasma* antibodies in transplant patients varies highly among countries and is again explained mostly by the differences in the incidence rates of toxoplasmosis (81). A recent Spanish case-control study showed that a negative serostatus prior to transplantation was the only independent risk factor for toxoplasmosis in SOT patients (125), confirming that primary infection is more frequently observed for these patients. In about half of those patients, toxoplasmosis was due to a D^+/R^- mismatch, emphasizing the need for primary prevention through both donor/recipient screenings and hygienic measures. In France, serologic screening is mandatory for the organ donor and is strongly recommended (in practice, it is always done) for the recipient, whereas in the United States, the serologic screening of donors and recipients depends largely on the transplant center. Determination of the serology of the donor is also routinely performed in 11 European countries (81). Knowledge of the serologic status of both the recipient and donor allows the start of primary chemoprophylaxis at the time of transplantation in the case of a mismatch, particularly for heart transplant patients. However, it was argued that in countries with a low *Toxoplasma* seroprevalence, such an occurrence is rare and that the systemic use of cotrimoxazole for the prevention of *Pneumocystis* pneumonia counterbalances the risk of primary *Toxoplasma* infection, at least in the first months following transplantation (154). If chemoprophylaxis is usually the rule for heart transplant patients in the case of a known mismatch, there is no consensus concerning the duration, which usually does not exceed 6 months. After prophylaxis is stopped, some heart transplant centers perform serological screening at 6-month intervals, with the aim to detect a potential seroconversion or reactivation. However, it must be acknowledged that serologic reactivation is rarely associated with clinical reactivation but may prompt further investigation when associated with clinical signs.

TMP-SMX (160 mg–800 mg) daily or three times a week is also the first-line drug regimen to prevent toxoplasmosis in SOT patients who also need to be protected against PCP (81, 154, 234). In the case of intolerance, pyrimethamine alone (25 mg/day) can be an alternative for *T. gondii* prophylaxis (234, 339), and aerosolized pentamidine can be used for *Pneumocystis* pneumonia. If prophylaxis must be stopped for any reason, the onset of clinical signs must prompt further investigations based on CT scans and parasite detection by PCR (248).

For HSCT patients, the risk of donor-acquired infection is negligible, as toxoplasmosis results mainly from a reactivation of a past infection in these patients. TMP-SMX is also the drug commonly used to prevent *Pneumocystis* pneumonia and toxoplasmosis in allogeneic transplant recipients, but its toxicity usually restrains its use after the first month following the graft. It must be kept in mind that patients receiving aerosolized pentamidine for *Pneumocystis* pneumonia prophylaxis are not protected against *Toxoplasma* infection and should be carefully monitored. Pretransplant serology should be performed for HSCT patients to evaluate the potential risk of reactivation, but serology is far less reliable after a graft due to profound immunosuppression. As the disease is rapidly progressive, systematic screening by repeated PCRs of peripheral blood has been proposed for the early weeks or months following transplantation (136, 215). In a study by Martino et al. (215), 16 of 106 (15%) patients had at least one positive PCR result, which prompted the initiation of preemptive therapy. Another prospective study found positive PCR results for 12.5% of *Toxoplasma*-seropositive HSCT patients; all were febrile, and the PCR signal disappeared after preemptive TMP-SMX therapy (44). Recently, Cavattoni et al. reported the usefulness of PCR to diagnose ocular and cerebral toxoplasmosis in three allogeneic HSCT patients who underwent pentamidine prophylaxis (52), underlining the heterogeneity of chemoprophylaxis practices in various countries. Repeated PCR testing can be also performed to monitor solid-organ transplantation in the case of a mismatch, especially for heart transplant patients, but biological diagnosis is guided mostly by the onset of clinical symptoms (39, 135).

Secondary chemoprophylaxis should be given after a first episode of severe clinical manifestations and should rely on a bitherapy. TMP-SMX (30) or pyrimethamine-sulfadiazine (25 mg–2 g daily or 50 mg–2 g three times a week) has been proposed as a maintenance therapy (260).

CONCLUSION

Substantial advancements in the field of *Toxoplasma* research have occurred in the past 20 years. Basic research in molecular and cell biology benefited from the development of high-throughput genomic and proteomic technologies and new imaging techniques. Another review will be necessary to summarize these advances. However, in the meantime, epidemiological studies also gained more importance. The emergence of *Toxoplasma* as a waterborne disease in several countries has stimulated environmental research. An ecological and integrated approach was developed for a better understanding of the complex circulation of *Toxoplasma* between its multiple hosts and the environment and, finally, of the risk factors for human infection. Thanks to the isolation and genotyping of strains from various animal species in different continents, the diversity of *T. gondii* was revealed. We now know that this unique species is not totally identical around the world. The pathogenic role associated with some of these genotypes has been well studied in experimental models. These models al-

lowed the detection of virulence-associated genes. Even if it is more difficult to formally establish the role of the infecting genotype in human toxoplasmosis due to the difficulty in isolating strains from patients and due to other pathogenesis-associated factors, such as host immune status and genetic background, it appears now that the description of clinical aspects of toxoplasmosis must be unraveled in the light of *Toxoplasma* genotypes and of their geographical distribution. However, the practical consequences of these epidemiological and genetic advances for diagnosis strategies and for the management of human toxoplasmosis still need to be evaluated. Available tools for the biological diagnosis of toxoplasmosis allow diagnosis in most cases, but the development of other biomarkers should be helpful for the most difficult cases, for instance, for ocular or cerebral localizations.

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Epidemiology of and Diagnostic Strategies for Toxoplasmosis

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Volume 25, no. 2, p. 264–296. Page 280, Fig. 5: The center box in the top row for the nonimmunized women should read "IgG−IgM−."

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